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BIOCHEMISTRY

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ELECTRON MICROSCOPY OF STRUCTURAL ORGANIZATION OF CHOLERA C PHAGE

Moscow VOPROSY VIRUSOLOGII in Russian No 6, Nov-Dec 80 (manuscript received 27 Mar 80) pp 735-740

[Article by B. M. Degtyarev, V. N. Milyutin, V. B. Grigor'yev, I. M. Alutin and Yu. I. Arutyunov, Rostov-na-Donu Scientific Research Institute of Plague Control]

[Text] Cholera phages referable to serovar [serological variety] IV [1, 2] consist of several lines: C-Saratov, C-London and phage IV of the (Mukerdzhi) group. The distinction of these bacteriophages is that they are highly specific to the classical biovar of Vibrio cholerae, so that they are used for differentiation between V. cholera of the classical biovar and the El Tor biovar.

The data obtained from the very first electron microscopic studies revealed that the particles of these phages have a large head with long fine process ending with a thickening [3-5]. The fullest morphological analysis of these phages was made in [2, 6, 7], where the morphological features of phage particles were one of the main tests for classification thereof.

We undertook the study of cholera phages by methods of electron microscopy on the level of structural organization of their protein membranes and nucleic acids [8], the parameters of which are also rather important taxonomic criteria. This is a continuation of such studies, and we submit here the results of studying the structural organization of cholera bacteriophage C-Saratov, referable to serovar IV.

Material and Methods

We used a pure line of cholera C phage from series K, No OBK 611 VNIPChI [All-Union Scientific Research Institute of Plague Control]. V. cholera strain 23 (1128) served as the host.

Recovery, concentration and purification of phage: A phage lysate was obtained by growing a mixture of bacteria and phages on cellophane membranes [9]. The culture of vibrions was grown in Marten broth (pH 7.6) for 5-6 h in a water bath at 37°C to a concentration of $1 \cdot 10^9$ cells/mℓ, and phage

was added to an end multiplicity of 0.025. The mixture was kept at 37°C for 15 min, then 0.1 mℓ of the mixture was spread over the surface of cellophane applied to a solid nutrient base, for which purpose we used Marten agar (pH 7.6) in Petri dishes. The mixture of vibrions and phage was incubated for 16-18 h at 35°C. The formed phage lysate was washed off with 0.01 M Na-K-phosphate buffer, pH 7.6 with 0.01% Na merthiolate. To remove bacterial residue, the phage lysate was submitted to low-speed stepped centrifuging at 3000-8000 r/min for 30 min. The phage was concentrated by high-speed centrifuging with partial purification with a 60% saccharose solution layered at the bottom of the test tubes in an SW-27 rotor of an L2-65B Spinco ultracentrifuge at 17,000 r/min for 1.5 h. The obtained sediment was suspended in phosphate buffer under refrigeration. Final purification of the phage was effected in a preformed linear CsCl density gradient under the same conditions.

In our subsequent work we used the extracted fractions containing whole phage particles or components thereof, which were dialyzed and checked under an electron microscope.

Phage DNA was isolated using two methods: phenol extraction and incubation of phage in 1 SSC. We performed deproteinization with purified phenol three times using the most conservative procedures to preserve the integrity of nucleic acid (NA) molecules. Extracted NA was dialyzed against a phosphate buffer and stored at 4°C. In another instance, heating a phage suspension in a titer of $1 \cdot 10^{10}$ particles per mℓ in 1 SSC at 65°C for 15 min served as the factor causing exit of DNA from the viral particles.

We used previously described methods [8] for electron microscopy of phage particles. A commercial Bal'zers unit was used for freezing and priming. Operating conditions were as follows: priming time 4 min at a pressure of $3 \cdot 10^{-6}$ torr, specimen temperature 100°C. An electronic gun was used to spray on a mixture of platinum and carbon at an angle of 30°; replicas were fixed with carbon and organic residue removed with 70% sulfuric acid for 6 h at room temperature. A JEM-1008 electron microscope, with instrument magnification of 50,000× was used for examination and photography.

The rotation method of Markham and Frey [10] was used to analyze radial symmetry of phage process discs, for which purpose a special revolving table was designed with precise fixation, used to take photographs. The image was amplified from the 5th, 6th, 7th and 8th positions.

The protein layering method [11] was used to obtain the phage DNA preparation. The hyperphase solution contained 1 M MH_4Ac (1 μM EDTA, pH 7.0), 100 mg/mℓ cytochrome C, 0.5 μg/mℓ NA from the tested phage and 0.1 μg/mℓ CdEl plasmid NA (as a standard). Layering was performed on the hypophase--0.25 M NH_4Ac (1 μM EDTA). In addition, we used the nonprotein layering method [12] for demonstration of structural distinctions of phage NA molecules, and it involved the following components in the hyperphase: 1 M NH_4Ac (pH 5.0), 5 μg/mℓ NA from the phage under study. A solution of 0.5 M NH_4Ac served as the hypophase (pH 5.0). The specimens were contrasted by circular spraying of Pt-C at an angle of 6° or dusting from one direction.

The preparations were examined and photographed with the electron microscope at magnification of 100,000×.

Results and Discussion

Figure 1*а* [photo not reproduced] illustrates the general appearance of a particle of cholera C phage. The distinction of this phage is that it has a long noncontractile process with developed adsorption system. The capsid of the phage is rather large, presenting hexagonal outline, like most of the phages studied, with the use of negative contrast. We determined the shape of the capsid by means of shading [13] and analysis of the obtained projections of shadows of phage particles.

We viewed essentially two types of shadows: tapered and trapezoid. Figure 1*д* clearly shows the head, tail of the phage and orientation of the projection of its shadow. It is perpendicular to the axis of the particle and has a trapezoid shape. A polyhedron of the icosahedron type can produce such a shadow if it is illuminated along the equatorial belt in a direction perpendicular to the 5th-order axis of symmetry. Particles of the dusted material, which encountered in their path three edges of the polyhedral head of the virus, formed a trapezoid shadow as they broke away from these edges, which would be impossible if the capsid had a different shape. The shadows could have different outlines if the spray of metal encounters the apex of the polyhedron on its way. The projection of the shadow would be tapered, like the one in Figure 1*г*. It is also possible to obtain two types of shadows if the phage particle is dusted along its axis. The projection of the shadow will depend on the location of the capsid or, more precisely, on how it will extend about the 5th order axis (axis of the particle), which part of the capsid participates in forming the shadow, the edge or apex (Figure 1*е*, *ж*).

The shape of the capsid is organically linked to the elements that form it, the nature of their arrangement. For this reason, the freezing-priming method was used to demonstrate the superficial structure of the head of the phage under study. Figure 1*б* illustrates capsids with visible morphological subunits on their surface. Of the images present, we can evaluate T--triangulation number--on the basis of the radius of the phage head and distance between the centers of adjacent capsomeres.

As we know, radius R of a sphere around an icosahedron and the distance A between the nearest apices of this polyhedron are linked by the following function:

$$A = \sqrt{2\left(1-\frac{1}{\sqrt{5}}\right)}R, \qquad (1)$$

in addition, A can also be expressed by T and a:

$$A = a\sqrt{T}, \qquad (2)$$

where a is the distance between the centers of adjacent capsomeres; hence:

$$T = 1{,}1\left(\frac{R}{a}\right)^2. \qquad (3)$$

Determination was made of the maximum diameter of C phage heads as a result of measurements of phage capsids along the 5th order symmetry axis and appropriate statistical processing of the obtained data: D_{max} = 81.2±308 nm, which yielded a value of close to 7 for T, in equation (3) with a = 15 nm.

Then, proceeding from the assumption that any shell of an icosadeltohedron with triangulation number T can be constructed of 12 pentamers and 10 (T = 1) hexameres, and the ensuring equation [14]:

$$Mu = 12 + 10(T - 1), \tag{4}$$

we calculated the number of morphological subunits constituting the phage capsid: Mu = 72.

Under an electron microscope, the isolated DNA molecules from the heads of the phage in question presented a linear appearance, with no circular or oligomeric forms. Of the two methods used to isolate phage NA, heating in 1 SSC at 65°C turned out to be the most sparing; the demonstrated molecules were virtually the same in size, but more convoluted, in contrast to the NA molecules isolated by phenol extraction. In all instances, incubation for 15 min led to complete removal of DNA from the phage heads (Figure 2б [photo not reproduced]).

Main structural parameters of cholera C phage

Method	Parameter	Value
Freezing-priming	Maximum capsid diameter, nm	81.2±3.08
	Distance between centers of adjacent morphological capsid subunits, nm	15±0.5
	Capsid triangulation number	7
	Quantity of morphological subunits forming capsid	72
Negative contrast	Length of process, nm	197.7±3.1
	Diameter of process, nm	14.5±0.5
	Number of discs forming process	36
	Thickness of single disk, nm	5.5
	Order of disk symmetry axis	6
	Quantity of morphological subunits contained in process	216
	Diameter of internal channel of process, nm	≈2
	Quantity of fibrils in adsorption system of process	3
	Length of fibrils, nm	109±1.2
	Length of distal filament of process, nm	51.5±5.29
	Molecular mass of phage DNA, dalton	$(68.16\pm1.63)10^6$

We estimated the length of C phage DNA molecules by comparing them to standard NA, in this case, Col E1 plasmid DNA which is 2.28 μm in length. With the ratio of 11.19, the DNA of the phage under study constituted 35.5±0.85 μm, which corresponds, according to linear density of DNA in the B form of 192 dalton/A, to a molecular mass of (68.16±1.63) 10^6 dalton.

Use of the protein-free method failed to demonstrate any structural distinctions in phage NA. The molecules were essentially rectilinear and elongated in one direction (Figure 2*a*). The diameter of the molecules, estimated from the length of the shadow, was close to 2 nm [15].

There is a rather flexible process, 197.7 nm in length, which negative contrast shows to be a tubular element with well-visualized transverse markings in the form of several discs (36), which in turn consist of subunits (see Figure 1*a*), joining one of the apices of the phage capsid. The absence of a neck and collar, which are typical for this group of phages [16, 17], is a distinctive feature of the C phage process.

Examination of fractions isolated in the course of purification enabled us to detect hexagonal elements in the form of discs (Figure 1*в*) that separated from the processes. The presence of an even-numbered symmetry axis (N = 6) in them was confirmed by restoring the image according to Markham (Figure 1*з*). This led to the assumption that the process discs consist of 6 subunits, so that with a known number of discs we could calculate the quantity of morphological subunits forming the entire process (216). The obtained data concerning the circular symmetry of the process supplement the results of freezing and priming, which demonstrated an angle of inclination of morphological subunits indicative of spiral symmetry of the phage process.

The process ends with an adsorption system with rather unique structure (Figure 1*е*). Its base is a disc that is located at a small distance from the process. It gives off three fibrils that end with thickenings in the form of suction discs [or suckers]. A fourth filament, which is distal and not in contact with the disc, is a continuation of the process. Such a structure cannot be referred to any of the three types of terminal structures of bacteriophages with noncontractile process proposed by A. S. Tikhonenko [17]. The distal end of the process of cholera C phage, which has elements of the second and third types of terminal structures, may apparently be a transitory form between them.

According to the results of this electron microscopic study (see Table), cholera C bacteriophage, with its characteristic features, is a typical representative of phages with noncontractile processes. This is indicated by the icosahedral phage head with triangulation number 7, which consists of 72 morphological subunits and contains DNA with molecular mass of (68.16±1.63)•10^6 dalton; it has a long, noncontractile process consisting of 216 morphological subunits ending with an adsorption system that is an intermediate form between the second and third types of terminal structures of phages referable to this morphological group.

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PROTOPLAST FUSION--A NEW METHOD OF OBTAINING RECOMBINANT STRAINS

Moscow ZHURNAL MIKROBIOLOGII, EPIDEMIOLOGII I IMMUNOBIOLOGII in Russian No 12, Dec 80 (manuscript received 5 Nov 79) pp 3-9

[Article by R. R. Azizbekyan, All-Union Scientific Research Institute of Genetics and Breeding Industrial Microorganisms, Moscow]

[Text] In recent years, a basically new technique is being used to obtain recombinant strains. This method, which was originally developed for plant cells and fungi [8, 23], was subsequently adapted for eukaryote and prokaryote microorganisms.

The method consists essentially of the following. Cells are changed into protoplasts, i.e., structures wanting in cell walls, by using lytic enzymes (lysozyme, zymolase,"helix" enzyme [?] and others). Then, in an osmotically stabilized medium in the presence of polyethylene glycol (PEG), the protoplasts fuse, and there may be more than two protoplasms that unite, i.e., fuse. The probability of protoplast fusion depends on a number of factors, including the surface charge and free energy of interaction. PEG, which lowers drastically the free energy of the cell surface, causes fusion of protoplasts. One can also lower the surface charge by adding calcium ions to the solution [16].

The mechanisms of physicochemical reactions and morphological transformations involved in the fusion process have not been identified. It is assumed that, at the first stage, there is formation of wide areas of contact between cytoplasmic membranes. Then the membrane structure disappears in the contact zone. At the last stage, there is complete fusion of genetic and cytoplasmic material of the protoplasts [18].

The above method of producing recombinant variants has a number of advantages: it is relatively simple, and it is possible to produce recombinant strains with multiple markers without loss of the two parent markers in counterbreeding, i.e., it is possible to rapidly build complex polyauxotrophs [19].

With the standard methods of transferring genetic information (transformation, transduction, conjugation), there is a substantially lower yield of recombinants than with the protoplast fusion method. What is particularly important is that the protoplast fusion method can be used when working with microorganisms, for which few or no methods of genetic analysis have been elaborated, i.e., when there are no effective means of exchanging genetic information.

New opportunities are revealed by using the method of fusion as a tool for producing interspecific recombinant variants. The superficial elements of the cell wall, where foreign DNA is subject to nuclease attack [30], is the first and substantial obstacle to insertion and expression of foreign genetic material. Use of protoplasts makes it possible to "bypass" the external nuclease barrier.

Genetic exchange, which occurs with protoplast fusion, is basically different from the known methods of transmitting genetic material. In the case of transformation, transduction and conjugation, only a fragment (or fragments) of donor parent chromosome is spliced into the recipient cell. Thus, until the recombination process is completed, the recipient cell is a heterogenote, i.e., it carries the complete genome of the recipient cell and fragment (or fragments) of the donor cell. In the case of protoplast fusion, one can find the complete sets of genomes of all parents involved in the fusion in the newly formed morphological structure, and when several parent strains are used there is a different probability of combination of genome sets.

A very important factor that affects the process of formation of recombinant variants is that, with fusion, there is also mixing of cytoplasmic material of parent strains. These distinctions of the protoplast fusion method have an appreciable influence on the qualitative and quantitative characteristics of the recombination process. The share of recombinants appearing as the result of protoplast fusion depends largely on the degree of regeneration of parent strains, and regeneration itself is a function of physiological and biochemical distinctions of parent strains, cultivation conditions, breeding media, etc. At the same time, no direct correlation is observed between the level of regeneration and yield of recombinant strains, i.e., the impression is gained that these two processes are independently determined. Moreover, we cannot rule out the possibility that the rate of regeneration of formed recombinant protoplasts (or structures) and entire protoplast population is also unrelated [14].

Although protoplasts retain the capacity to form new cell walls, this potential capacity is not necessarily realized. Some types of cells require additional specific factors for regeneration.

Reversal of protoplasts to the cell form undergoes three stages: 1) growth of protoplasts; 2) regeneration of cell walls; 3) protoplast reversion proper, during which there is total restoration of all cell structures and functions [27]. Regeneration of cell walls is a de novo process, i.e., it does not require residual elements of cell walls as a matrix for biosynthesis.

The recombinants formed as a result of protoplast fusion are heterogeneous. Clonal analysis of the segregant progeny revealed a number of anomalies in marker distribution in the latter.

In this survey, we shall discuss the main works dealing with production of recombinant variants of microorganisms by the protoplast fusion method, as well as analyze the literature dealing with protoplast fusion in fungi, since there are common patterns to this process in microorganisms and fungi. We devoted attention mainly to the genetic aspects of producing recombinants by the fusion method.

The first work on producing recombinant strains of microorganisms by the protoplast fusion method, with the use of PEG, was done by two teams of authors with sporulating bacteria [10, 32].

The choice of B. megaterium as the object was due to the fact that, at the time the study was conducted, it had not demonstrated any means of transfer of genetic information (let us note that it was recently demonstrated that generalized transduction is possible in B. megaterium [40]). Two auxotrophs of B. megaterium were used in the fusion experiments: Arg^- Leu^- (which requires arginine and leucine for growth on minimum medium) and His^- Trp^- (auxotroph for histidine and tryptophan). The protoplasts of both strains were treated with PEG; the mixture was plated on minimum agar without growth factors. The incidence of colonies on minimum agar (primary colonies) constituted 10^{-6}-10^{-3}, and their number did not decrease in the presence of DNAase. It was interesting to determine the composition of the primary colony, genetic identity and stability of cells in the colony, i.e., to make a clonal analysis of the offspring. Without going into the methodological details of this analysis, let us mention that the offspring of primary colonies consisted of the following subclasses: 1) colonies yielding a mixed population, in which the prototroph forms were unstable for several passages; 2) colonies whose offspring consisted of a mixed population of stable cells of different types; 3) colonies whose offspring consisted of cells of one type (prototroph or recombinant). In some cases, the primary colonies were formed as a result of physiological complementation of auxotroph cells, and upon further incubation they underwent segregation. The heterogeneity of primary colonies formed on selective media was also demonstrated in strains of B. thuringiensis, the producer of crystalline entomocidal toxin [1]. Along with stable recombinants, there were clones which became genetically stable after several generations. Of interest is the fact that recombinants with stable genotype could arise from unstable diploid forms in the passage process. The nature of occurrence and persistence of long-existing unstable variants (probably polyploids) is not yet clear; however, their appearance stresses the complexity [difficulty] of using the protoplast fusion method for genetic mapping, although it does not rule out the possibility of using it as a tool to build recombinant strains.

The absence of genetic maps for B. megaterium and B. thuringiensis did not enable the authors to pursue a comparative analysis. Such an opportunity was provided with the use of B. subtilis. Schaeffer et al. [32] used polyauxotrophic strains, each of which "carried" three auxotrophic markers. The authors were unable to obtain complete prototrophs, i.e., recombinants for all six genetic markers, and this was not attributable to the need of more (4) crosses for recombination. The reciprocal location of genetic markers involved in the recombination process was found to be a more important factor affecting the number of partial prototrophs. Evidence of fusion of parent strain cytoplasm was obtained in experiments where a lysogenic strain was used as one of the parents. With protoplast fusion there was no zygotic induction of prophage, and there was no change in number of recombinant colonies. Consequently, unlike the conjugation process where only genetic information--prophage--was inserted in the recipient cell, with the use of protoplasts there was also fusion of parental cytoplasmic material, as a result of which the repressor also provides for immunity in the de novo structure of fused

protoplasts. The work of Levi et al. [26] confirmed the possibility of fusion of cytoplasmic material of parent strains.

Treatment with streptomycin of an antibiotic-sensitive strain of protoplasts did not affect the process of formation of recombinants if a streptomycin-resistant strain was used as the other parent. When protoplasts of the sensitive strain, which were killed by streptomycin, fused with protoplasts of the antibiotic-sensitive parent no recombinants were demonstrable. The authors assumed that, with inactivation by streptomycin, there is accumulation in cytoplasm of toxic material in a sufficient amount to kill the sensitive partner when there is fusion of protoplasts.

The dual direction of transfer of genetic material is another substantial distinction of the protoplast fusion method, as compared to other methods. The main principle of bacterial genetics implies polarity of transfer, i.e., the same direction. Fodor et al. [11], who used heat-inactivated protoplasts, demonstrated that transfer of genetic material is possible in both directions with fusion, i.e., both parents can serve as recipients. Heat inactivation of protoplasts makes it possible to create a direct system for selection of recombinants, in which a prototrophic strain can be used as one of the parents. The heat-inactivation procedure has also been used to optimize protoplast formation and selection of recombinants of Micromonospora, which produces the antibiotic, gentamycin [22].

We mentioned above that cultivation conditions and recombinant selection play a substantial role in realization of the recombination process with fusion of protoplasts. The basic principle of genetic analysis using classical methods (transformation, transduction, conjugation) is the assumption that all genotypes on appropriate media have equal opportunity for expression, i.e., formation of colonies, and the number of formed colonies of the recombinant type depends only on reciprocal location of genetic markers and distance between then. In the case of using the fusion method, anomalies are demonstrated in distribution of unselected markers. Clone analysis of offspring of primary colonies, when they are further incubated on media with different growth factors, was pursued by Fodor and Alfoldi [12]. In several instances, the authors found complete coincidence of theoretically expected and experimentally obtained number of colonies of the appropriate recombinant genotype. At the same time, when histidine and arginine or histidine and leucine (factors needed for growth of parent strains) were added to the selective medium, there was a drastic decrease in number of colonies, as compared to the estimates. Serine, tryptophan and certain amino acid combinations had an inhibitory effect when another pair of parent strains was used, and serine suppressed completely the growth of possible recombinants.

The primary selection medium also had an effect in the case of protoplast fusion in fungi. Depending on the composition of the medium, either haploid strains (on a good medium) or heterozygotes were demonstrable. However, of greater interest is the fact that with subsequent segregation there were auxotrophs carrying auxotrophic or morphological markers of only one of the parents. Such asymmetric segregation apparently indicates that one of the parents may play the role of acceptor, i.e., dominate in the fusion process

[24]. Analogous asymmetry of segregation of unstable heterozygotes was found in B. thuringiensis protoplast fusion [1].

Thus, the heterogeneity of primary colonies and anomalous marker distribution are indicative of some degree of unpredictability of the results of genetic analysis of marker linkage when producing recombinants by the protoplast fusion method. This is also indicated by the results of analysis of segregation of nonselective markers among recombinants with multifactor hybridization of Streptomyces coelicolor [19]. The authors demonstrated "weaker" linkage of genetic markers, i.e., an increased incidence of recombination per unit of genetic map. Such "weakening" of marker linkage could be the result of either more complete diploidy of structures formed in the fusion process, or a larger number of recombination rounds. Let us mention that there are convincing genetic and microscopic data concerning fusion of more than two protoplasts [13, 29]. Thus, Hopwood and Wright, who used auxotrophic mutants [20], demonstrated that it is possible for recombination of markers of four parent strains involved in the fusion process, and the incidence of recombinant formation constituted 4.9×10^{-7}; when fewer parents were involved in the recombination process, there was an increase in incidence of recombinants ($4\cdot10^{-4}$ for three parents and $2.8\cdot10^{-2}$ for two), since it is a function of probability of protoplast fusion. Several types of parents could also be involved in the fusion process in fungi, although the incidence of recombination dropped [36].

Several attempts have been made to optimize conditions for obtaining recombinants, as well as to determine the correlation between processes of cytological fusion of protoplasts and recombinant yield. It was possible to demonstrate the stimulating effect of UV [ultraviolet] radiation on the yield of recombinants for two microorganisms. In experiments with S. coelicolor A3 [2], UV irradiation of protoplasts increased by 10 times the incidence of recombinants, as well as share of colonies containing recombinants [21]. Exposure of B. thuringiensis protoplasts to UV radiation increased the relative share of clones that yielded up to 60-90% recombinant offspring [1].

Changing cultivation conditions after treating protoplasts with PEG was also found to be an important factor in improving the effectiveness of the protoplast fusion process: incubation in a medium with nutrient broth increased by 100 times the number of demonstrable protoplast contacts [31]. The authors of that study proposed a clever method for counting the cases of protoplast fusion, using suppressor (sus) mutants of 105 phage of B. subtilis. Su^- bacterial strains, which were lysogenous for different sus mutants of phage, were induced, changed to protoplasts then, after fusion, the mixture was plated on sensitive cultures (Su^+ or Su^- strains, i.e., those with and without suppressor). Cytological fusion of protoplasts was demonstrated by production of negative colonies, since they could arise either as the result of only recombination (negative colonies of phage were demonstrable in a subculture of Su^- sensitive culture), or as a result of two events--recombination and complementation of phage sus mutants (negative colonies demonstrable on an Su^+ subculture). As was to be expected, there were considerably more negative phage colonies on the Su^+ subculture than Su^- strain subculture, since in the latter case there was imposition of restrictions related to the

process of recombination of sus mutants of the phage. Thus, evaluation of protoplast fusion according to number of recombinants does not reflect the effectiveness of the fusion process, although there is in essence a correlation between probability of fusion and recombinant yield.

Let us consider some aspects of utilizing protoplasts. Drastic widening of the range of microorganisms, on which use of the fusion method made it possible to transfer genetic information, should be considered one of the most promising directions. Recombinant strains were obtained of sporulating bacteria [1, 10, 32], Gram-negative bacteria of the Enterocoli group [2], actinomycetes [4, 19, 20], fungi [3, 8] and yeast [33-36, 39]. The most valuable factor is that this method makes it possible to obtain polyploid strains, as well as intraspecific and interspecific recombinants [9, 17, 38]. None of the previously developed methods of transmitting genetic information had been used for such a wide range of prokaryote and eukaryote organisms. Let us also mention the applied aspect of using the protoplast fusion method. In particular, it can be used extensively to obtain recombinant strains of streptomycetes. Let us recall that up to 95% of the antibiotics used and a number of enzymes are synthesized by streptomycetes. However, there are several factors that restrict the use of conjugation crosses to obtain strains with recombinant features. The protoplast fusion method developed by Hopwood et al. [19] is presently used to obtain intraspecific and interspecific hybrid strains. Presence or absence of sex plasmids in the strains does not affect the incidence of recombinants. The level of recombination, like the incidence of multiple recombinants, is considerably higher than with conjugation hybridization.

The success of using the protoplast fusion method for practical breeding, i.e., to obtain producers with features of practical value, may depend on the following conditions: choice of parent strains, optimization of conditions of selecting recombinants with consideration of selective pressure in the process of protoplast regeneration, choice of selective media, selective pressure in the process of industrial cultivation. The first practical results have been obtained: a strain with increased capacity for penicillin synthesis was obtained with the use of protoplasts of auxotrophic mutants of Penicillium chrysogenum [28]; this feature was related to increase in spore size in the recombinant strain.

With fusion of P. chrysogenum and P. notatum, heterokaryons were isolated at an incidence of $0.8\text{-}58.5 \cdot 10^3$/mℓ (depending on the pair of parent strains). In most cases, the heterokaryons were diploids, the size of their spores and DNA content exceeded these parameters in haploids. Thus, the mean spore diameter was 2.95 and 3.6 for haploids and diploids, respectively, and their DNA content constituted 2 and 4.3 μg/10^8 spores [3]. A similar increase in genetic material was also observed in fused structures of yeast. Thus, while the mean DNA content of haploid cells constituted 0.0405-0.0503 pq/mℓ, it was twice as high in fused structures--0.0959-0.1182 pq/mℓ [33]. Diploids, triploids and tetraploids can be obtained in yeast by the protoplast fusion method [36]. At the same time, the hybrid recombinant strains may be aneuploid, i.e., contain the entire set of chromosomes of one of the parents and one or several chromosomes of the other parent [37, 38]. Isozyme polymorphism may be observed in recombinants obtained by protoplast fusion, i.e., the

recombinants may present an enzyme spectrum with both hybrid features and parameters corresponding to enzymes of parent strains [25].

Let us briefly consider some of the potential possibilities for utilizing protoplasts. We mentioned already the advantages of this method for introduction of foreign genetic information. Transformation of plasmid DNA protoplasts is also quite promising. The mechanism of action of PEG in transformation of protoplasts is not clear. Perhaps, interaction of PEG with cell membranes renders them more permeable to DNA, although we cannot rule out the possibility that DNA acquires a more compact form in the presence of PEG [5]. Chang and Cohen [6], who used B. subtilis protoplasts, obtained exceptionally high figures for transformation of plasmid DNA--up to 80% of the cells were transformed, and the effectiveness of transformation constituted $4 \cdot 10^7$ transformants per μg DNA. A high level was also obtained in transformation with protoplast plasmid chimera in yeast [15]. Chimera plasmids contained bacterial plasmid pCR1, the yeast plasmid and yeast gene ura 3. After transformation by the chimera plasmid, gene ura 3 was maintained in transformed cells as a cytoplasmic element.

Successful transformation of transmissible and nontransmissible plasmids was demonstrated in E. coli protoplasts [41].

Bibb et al. [5], who used plasmid DNA scp 2, demonstrated that transformation of streptomycetes protoplasts was possible (the transformation effect was assessed according to "lethal zygosis"). There was a direct correlation between concentration of plasmid DNA in solution and number of transformed cells.

Let us also mention the possibility of protoplast transformation by means of DNA contained in liposomes [7]. Although protoplasts of plant cells were used in the cited study, there are no basic restrictions to use of this method for bacterial protoplasts. It should be noted that incorporation of DNA, like that of biologically active substances--enzymes, hormones, etc.--in liposomes suggests a very promising route for rapid transfer of these compounds.

Thus, we have explored the possibility of using protoplasts to obtain recombinants. Producing cell protoplasts, with subsequent PEG treatment, is a methodologically simple procedure, and it can be used for recombinant strains.

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PROTECTIVE STRUCTURAL ANTIGENS OF VIRUSES USED TO DEVELOP NEW TYPE OF CHEMICAL VACCINES

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[Article by V. I. Kostyukov, A. I. Yarov and Ye. G. Zezerov]

[Text] Live and killed vaccines are used extensively in current specific prevention of viral infections. In many cases, the use of a number of vaccines provides reliable protection. However there are also some flaws inherent in live and killed vaccines, the most significant of which are postvaccinal, clinically marked complications, allergic changes in the macroorganism and cytogenetic damage--chromosome aberrations in various cells, including those of bone marrow [1]. In addition, each live and killed vaccine has its own specific flaws. For this reason, the current level of development of effective agents does not eliminate the problem of improving them and developing a new type of chemical vaccines, molecular vaccines, which contain no immunologically inactive [inert] proteins of virus and cells, as well as nucleic acids apparently responsible for chromosome aberrations.

The feasibility of developing refined immunoprophylactic agents is based on data, which were obtained in the last 5-10 years, concerning the functional role of different structures and polypeptides of viral particles. At the present time, basically new vaccines are being developed intensively from viral components in a number of laboratories. These are so-called protein, chemical, molecular, subunit or split vaccines (without going into details about the terminological distinctions). We should refer to the resolution adopted by the 16th All-Union Congress of Microbiologists and Epidemiologists: "To intensify development of theoretical and applied aspects of the problem of obtaining highly immunogenic, molecularly homogeneous chemical vaccines with minimal reactogenicity and guaranteed safety, on the basis of using gradient centrifuging, chromatography, membrane ultrafiltration and other methods" [2]. For the above vaccines, only part of the structural components of viral origin is used, so that such vaccines must have a number of advantages over live or killed ones. First of all, this refers to the strict specificity of antibodies produced only against the required antigenic determinants. The absence of foreign nucleic acids also guarantees the absence of genetic changes after immunization. The possibility of developing complex agents against several infections and rapid production of vaccines for newly isolated strains or viruses also constitute important advantages of molecular vaccines.

Properties of protective structural antigens of animal viruses

Family or genus of virus	Virus species	Polypeptides in viral membrane	Properties of protective antigens: carbohydrates	molecular mass, dalton	number of polypeptides & their molecular masss (in parentheses)	neutraliz. antibody induction	animal protection against infection	other functions and properties	Literature source
Picornavirus	Coxsackie B3	NM	—	NT	1 (28 000)	+	NT	Precipitinogen	[7]
	FMD virus	"	—	34 000	1 (34 000)	+	Active	Inactivated by trypsin	[8—10]
Togavirus	VEE virus	2	+	59—56 000	1 (59 000—56 000)	+	Active & passive	HA	[11]
	Chikungunya virus	NT	+	NT	NT	+	Passive	HA	[12, 13]
	Sindbis	2	+	NT	1 (50 000—47 000)	+	NT		[14]
	Japanese encephalitis virus	1	+	NT	1 (58 000)	+	NT	HA	[15]
Rhabdovirus	Rabies virus	1	+	400 000	1(tetramer80 000)	+	Active	HA	[16, 17]
	VSV	1	+		1 (69 000)	+	"		[18]
Orthomyxovirus	Influenza virus	3—4	+	150 000	2 (50 000 & 30 000)	+	"	HA	[19—21]
			+	240 000	1—2 (50 000)	—	"	NA	
Paramyxovirus	Sendai	2	+	NT	1 (65 000)	+*	Passive	HA, NA	[22]
			+	NT	1 (53 000)	+	"	Inhibits hemolysis	[23]
	Newcastle disease	2	+	NT	1 (65 000)	+	NT	HA, NA	
	Mumps virus	2	+	NT	1 (75 000)	+	Active		[24]
Retrovirus	Rauscher mouse leukemia	3	+	NT	2 (71 000 & 69 000)	+	NT		[25, 26]
	Friend mouse leukemia	2	+	NT	1 (71 000)	+	NT		[27]
	Simian sarcoma	NT	+	NT	1 (70 000)	+	NT		[28]
	"Furry" simian virus	NT	+	NT	1 (70 000)	+	NT		[29]
Adenovirus	Adenovirus type 5	NM	+	360 000	1 (trimer 120 000)	+	Active	Hexone	[30]
Herpesvirus	Herpes simplex	3—12	+	60 000	1 (60 000)	+	"	Filament	[31]
Poxvirus	Vaccinia	To 11	+	123 000	1 (123 000)	+	NT		[32, 33]
			+	⩾200 000	2 (40 000 & 36 000)	+	NT	E complex	
			—	NT	1 (58 000)	+	NT	Membrane tubule protein	
Unclassified	Hepatitis virus B	2**	+	NT	1 (12 000)	+	NT		[4, 34]
			—	22 000	1 (22 000)	+	NT		

*Antiserum to Sendai virus GP-65 presents neutralizing activity only in the presence of antibodies to rabbit γ-globulin.

**Surface antigen of hepatitis virus B serves as the source of protective antigens.

Note: Active protection--protection against infection of immunized animals; passive--protection with administration of antiserum or γ-globulin to protective antigen upon subsequent infection.

Key: NT) not tested NM) no membrane HA) hemagglutinin NA) neuraminidase

It should be mentioned that molecular vaccines have already been obtained for some bacteria, and they are used in both experiments and practice [3]. Unlike the question of bacterial vaccines of a new type, the problem of molecular viral vaccines is not covered sufficiently in the Soviet literature. There are works only regarding some groups of viruses [4-6]. Expressly this circumstance served as grounds to make this survey.

Thanks to development of methods of purifying viruses and separating them into components it has become possible to isolate the different structural proteins of virions and determine their functions, including the capacity to induce immunity in animals. Investigation of the properties of protective antigens is the theoretical basis for developing molecular vaccines. The Table lists data from the literature concerning the properties of protective structural antigens of different RNA- and DNA-containing viruses. This table shows that one and, less often, two structural proteins have a protective function. The protective antigens of most viruses are glycoprotein (GP) molecules, with the exception of picornaviruses and adenoviruses, in whose virions no GP is demonstrable. In addition to GP antigen, in vaccinia virus the production of neutralizing antibodies is induced by nonglycosylated protein, while the data concerning involvement of GP in determining the immunogenicity of hepatitis virus B are contradictory [4, 34].

It is deemed expedient to dwell in greater detail on questions related to the structure of GP and functional role of carbohydrate and protein parts of biopolymer molecules. At one time, intensive studies of erythrocytes and bacteria established that the terminal carbohydrates of glycosylated proteins determine the antigenic properties of group substances of blood and certain bacteria [35]. For the latter, there are vaccines based on surface polysaccharides. As a result, the problem of function of carbohydrates contained in viral particles also requires experimental solution.

To describe viral GP briefly, it should be noted that their qualitative composition differs little from that of other organisms. The principal carbohydrates are mannose, galactose, fucose, sialic acids, glucosamine or its acetylated derivatives [36-39]. Galactosamine and, perhaps, glucose are present in some viruses [37]. There are no sialic acids in orthomyxoviruses and paramyxoviruses. Total carbohydrate content of togaviruses, myxoviruses and rhabdoviruses ranges from 5 to 15%. Most of the carbohydrates are bound with the protein of the GP molecules and only an insignificant part with lipids [40].

Studies of the effect of a host cell on carbohydrate composition of viral GP revealed that there was some dependence of carbohydrate composition on cells on which the virus was cultivated [36, 39].

Studies of superficial GP of togaviruses and hemagglutinin of myxoviruses by means of proteolysis followed by gel filtration and analysis of glycopeptides revealed that the latter contain carbohydrate chains of two types, one of which has all of the carbohydrates inherent in viral GP (the chains are designated as A and B for togaviruses I and II for myxoviruses), while the other has mannose and glucosamine [41, 42]. In chains of influenza virus hemagglutinin,

fucose occupies only a terminal position, while galactose and mannose can be in any part of the carbohydrate chain [43]. The carbohydrate chains of the main structural GP of vesicular stomatitis virus (VSV), which is characterized by the presence of chains of one type but with different amounts of N-acetylneuraminic acid [44], were identified by similar methods of chemical and enzymatic degradation. There are two or three branches going from the branching point represented by mannose, and they end with N-acetylneuraminic acid. The carbohydrate part of the molecule is connected to the protein part by means of a linkage between N-acetylglucosamine and asparagine. Interestingly enough, the carbohydrate chains are similar to VSV chains in Semliki Forest virus [45].

Diverse methodological approaches are used to determine the functional role of the carbohydrate and protein parts of the viral GP molecule. For example, treatment with a mixture of glycosidase leads to reduction of infectivity and hemagglutinating activity of Semliki Forest virus; treatment of the virus with proteases reduces infectivity, hemagglutinating activity and destroys surface antigens [46]. Interesting results were obtained in experiments with avian myeloblastosis and avian sarcoma viruses [47]. A comparative study of GP-85* of two viruses revealed differences in responsibility of the carbohydrate and protein parts for expression of antigenic properties. Data on selective effects of a mixture of glycosidases or proteases indicate that antigenicity is determined by the carbohydrate part of GP in the virus of avian myeloblastosis and by the protein in avian sarcoma virus. Periodate oxidation of the carbohydrate of hepatitus virus B GP leads to loss of activity of two (a and d) out of four antigenic determinants [48].

Thus, on the basis of existing data, it is difficult to settle unequivocally the question of the role of carbohydrate and protein parts of the GP molecule in manifestation of antigenic properties. Nevertheless, most researchers tend to believe that carbohydrates are at the least necessary for complete expression of the main properties of viral GP.

With regard to viral protective antigens, it should be noted that the range of molecular masses is quite broad--from 12,000 to 200,000 dalton. However, one must take into consideration the distinctions of methods of isolating and purifying different viruses, which permit preservation of protein structure in a relatively unadulterated state (GP of vaccinia virus, retroviruses, rabies virus, antigens of type 5 adenovirus) in some cases, whereas in others lead to destruction to individual polypeptides with restoration of disulfide bonds of antigens (Venezuela equine encephalomyelitis (VEE), herpes, foot and mouth disease [FMD] and hepatitis B viruses).

All isolated protective antigens of virions occupy an external position, and most often they form diverse excrescences on the membrane (spurs, tubules, etc.). Such localization has been proven by several methods, the most popular ones being superficial labeling with radioactive iodine, catalyzed by lactoperoxidase, and changing protective antigens to a soluble state by treating the virion with various solubilizing agents.

The relatively easy change of surface antigens to a soluble state served as the methodological basis for isolating protective antigens for most of the

*Here and in the following, the digits refer to molecular mass in kilodaltons.

viruses listed in the Table. As a rule, nonion detergents NP-40 [15, 32, 33], Triton X-100 [14, 16, 17, 22, 23], Triton N-101 [18] and tweens [12, 22, 27] are used.

Use of nonionic detergents is attributable to the fact that, in the concentrations used, they change into solution surface proteins with preservation of their tertiary and, perhaps, quaternary structures. In some cases, solubilization is obtained by treating virions with concentrated urea solutions [7, 9]. Subsequent purification of different proteins is done by high-speed centrifuging [22, 32, 33], centrifuging in gradients of saccharose [7, 23] or cesium chloride [12, 13, 15], various chromatographic methods [18, 24-26, 28], isoelectrofocusing [16-18] or a combination of the above methods.

Electrophoresis in polyacrylamide gel, in the presence of sodium dodecylsulfate, was used to isolate protective antigens of FMD [8], VEE [11], herpes [31] and hepatitis B [34] viruses. This is associated with breakdown of viral proteins mainly to polypeptides. The areas of unstained and unfixed gel corresponding to individual polypeptides are cut out, the obtained segments are homogenized and polypeptides given to animals in the form of gel homogenate or in an eluted state.

Protective antigens isolated by the above methods are usually capable of induction of neutralizing and other antibodies in animals. At the same time, it is not always possible to retain immunogenic activity. For this reason, whatever the method used to isolate structural antigens of viruses, it is necessary to check the biological activity of the obtained preparations.

Determination of capacity to induce production of virus-neutralizing antibodies when given to laboratory animals is the principal method of assessing the protective function of the antigens in question. Humoral neutralizing antibodies play a significant role in specific antiviral immunity, and for a number of infections there is a quantitative correlation between level of neutralizing antibodies in blood serum and degree of protection of the animal against infection. In other cases, the level of neutralizing antibodies cannot always reflect quantitatively the degree of systemic immunity of the animal, but is indicative of immunological changes in the organism.

Virtually all viral proteins listed in the Table induce production of neutralizing antibodies when animals are immunized, and in some cases they do so in very high titers. For example, antisera to VSV GP [18] and vaccinia virus [32] have titers of 1:60,000 and 1:12,000, respectively. Antisera to protective antigens of many viruses have titers in the range of 1:500-1:3000. Such high titers are attributable to the fact that authors use multiple injections of significant amounts of antigen in complete and/or incomplete Freund adjuvant. Sometimes, a high level of neutralizing antibodies is also obtained with a single injection of antigen [12]. However, it is difficult to detect induction of neutralizing antibodies in some viruses, and then only with multiple injections of large doses of antigen with adjuvant. For example, an antibody titer of 1:320 is obtained with multiple immunization of rabbits with GP-50 of Sindbis virus after 14 injections of a mixture of antigen and adjuvant [14], which is related, in the authors' opinion, to instability of the preparation in the course of storage. Nevertheless, the protective

antigens of most viruses studied provide for production of a rather high level of neutralizing antibodies, when properly administered to animals.

The most reliable criterion of protective activity of structural proteins is the presence of protection in animals after immunization with antigen, as well as after administration of antibodies in the form of antiserum or γ-globulin. The protective effect of serum antibodies to protective antigens has been demonstrated in mice infected with Chikungunya, VEE, Sendai viruses and type 5 adenovirus [11, 13, 22, 30]. In the case of Sendai virus, in addition to γ-globulin to GP-53, which contains neutralizing antibodies, protective properties were present to a lesser degree in γ-globulin against hemagglutinin-neuraminidase (GP-65) which does not neutralize the virus in vitro. This indicates that the absence of neutralizing antibodies is not necessarily a criterion of absence of protective function.

The protective effect of protective antigens in the case of active immunization was assessed in FMD, VEE, VSV, rabies, influenza viruses and type 5 adenovirus [8, 10, 11, 16-18, 30]. The protective antigens of these viruses protected animals against infection. A comparison of immunogenicity of protective antigens and inactivated viruses revealed that more protective antigen protein than viral protein is required for FMD virus and adenovirus type 5 to induce the same level of animal protection [8, 30]. In the case of VSV and VEE, the doses of protective antigen and inactivated virus are comparable [11, 18], whereas for rabies virus the dosage of antigen that protects 50% of the mice against death after intracerebral infection constitutes 0.009 μg GP, as compared to 1.63 μg whole inactivated virus [16].

The differences in relative immunogenicity of protective viral antigens may be due to many causes (method of isolation and degree of unadulteration of antigen, distinctions of pathogenesis and immunogenesis, stability of antigen, etc.). We were impressed by the fact that nonglycosylated proteins of the membrane-free FMD virus and type 5 adenovirus have a less marked protective effect, whereas the GP of VSV, VEE and rabies virus membranes are more active. This could be related to the greater stability of GP molecules, in particular with regard to proteolysis, due to the side carbohydrate chains and, consequently, longer circulation in the organism. Moreover, in the course of isolation there could be aggregation of GP molecules due to presence in them of hydrophobic ends which supposedly affects the process of formation of immunity [49].

The protective antigens of influenza virus--hemagglutinin and neuraminidase--have been studied in rather great detail; they form two types of excrescences on the virion surface: one is a hemagglutinin dimer and the other is a neuraminidase polymer [40]. Separate studies of hemagglutinin and neuraminidase revealed that hemagglutinin plays the leading role in induction of neutralizing antibodies, whereas antibodies to neuraminidase (like antibodies to hemagglutinin) prevent the release from cells of de novo synthesized daughter virions [21].

At the present time, molecular (split) [segregated] influenza vaccines have been developed, which contain hemagglutinin and neuroaminidase as the immunizing factor. Several experimental batches of such vaccines have passed trials on animals and volunteers. A study of influenza vaccine against A/England 42/72

virus, which was obtained by treating purified virions with Triton X-100 in a free state and sorbed on aluminum hydroxide, revealed that both vaccines are capable of causing production of a similar level of virus-neutralizing antibodies in hamsters and rabbits, but only the sorbed vaccine protects them effectively against experimental infection [50, 52]. Intramuscular injection of an analogous sorbed vaccine against A/Port Chalmers/73 to volunteers revealed that there were no toxic reactions, the mean geometric titers of neutralizing antibodies increased from 1:5 to 1:196 and there was induction of local antibodies (IgA) in washings from the nose of 45% of the subjects. After infection with homologous virus, there was a high degree of protection: 1 case of disease in vaccinated subjects versus 14 in the control group [51]. Studies on volunteers of the dependence of efficacy of sorbed vaccine derived from influenza A/Port Chalmers/73 virus on antigen dosage revealed that resistance to attenuated WRL-105 virus was observed 4 weeks after vaccination in subjects given at least 25 IU vaccine [53]. Kunz compared the efficacy of killed virus and molecular influenza vaccines (sorbed and nonsorbed) used for subcutaneous vaccination of volunteers [54]. A study was made of A/Port Chalmers/73 monovaccine and A/Port Chalmers/73, A/Scotland/74 and B/Hong Kong trivalent vaccine. It was shown that molecular vaccines are the least reactogenic and quite effective, particularly in high doses; but, unlike the results obtained on animals, use of aluminum hydroxide as adjuvant caused a reduction of antibody production in man.

The data obtained from studies of the properties of molecular influenza vaccines indicate that they are areactogenic, induce specific antiviral immunity and can be used to vaccinate humans.

At the present time, the technology of producing molecular vaccines against FMD, rabies, measles, rubella viruses, adenoviruses, etc., is being developed.

The main problem in the above work is to obtain large amounts of viral structural antigens. In this respect, the use of purified viruses as a source of antigens is important, not only from the standpoint of possibility of obtaining protective antigens, but use of the latter as marker polypeptides in developing new preparative methods that provide for an increased yield of protective proteins. One of these methods could be extraction of viral antigens from infected cells and tissues, at the expense of both the virions proper and virus-induced soluble antigens. Use of such a method could provide for fuller extraction of virus-specific protective antigens and bring us closer to an economically realistic technology. We cannot rule out other approaches to solving the problem of mass production of protective antigens. When the exact structure of the immunogenic determinant of protective antigen is defined, it can be synthesized chemically or in an acellular protein-synthesizing system, provided the appropriate gene is added to it as a template. In the case of synthetic reproduction of molecules, the task may be simplified if it is determined that only some parts of the protective antigen molecule have protective properties as, for example, has been shown for FMD virus. Namely, peptides with molecular mass of less than 12,000 dalton, obtained by separation of the main polypeptide with cyanogen bromide stimulate, just like the whole molecule, the production of neutralizing antibodies and partially protect animals against infection [10].

Thus, the advances made in the study of protective structural antigens of viruses and development of molecular vaccines on the basis thereof lead us to expect that, in the future, new harmless and effective vaccines will be used in immunoprophylaxis, which contain only specific viral components as the immunogenic element.

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FACTORS AFFECTING PLAQUE FORMATION BY LASSA VIRUS IN VERO CELLS

Moscow VOPROSY VIRUSOLOGII in Russian No 1, Jan-Feb 82 (manuscript received 31 Mar 81) pp 57-60

[Article by I. S. Lukashevich, A. D. Vasyuchkov, R. V. Mar'yankova and V. I. Votyakov, Belorussian Scientific Research Institute of Epidemiology and Microbiology, Belorussian Ministry of Health, Minsk]

[Text] Vero cells are used extensively for titration of pathogenic Lassa, Machupo and Junin arenaviruses by the plaque method [1-3]. However, there are only isolated studies of the effects of adsorption time, pH and polycations on effectiveness of plaque formation by Machupo and Junin viruses in this line of cells [2-4]. We did not encounter such studies with reference to Lassa virus in the available literature.

We studied here the effects of adsorption time, temperature, pH, polycations, trypsin, dimethyl sulfoxide (DMSO) and other factors on effectiveness of plaque production (viral titer and plaque size) by Lassa virus in Vero cells.

Material and Methods

Virus: We used Lassa virus (Sierra Leone). The virus was cloned twice from plaque to plaque in Vero cells and reproduced as previously described [5]. The initial viral titer constituted 10^6 PPU [plaque-producing units]/mℓ.

Plaque method: To titrate infectious activity of Lassa virus by the plaque method, Vero cells were cultivated at the bottom of scintillation vials. We used our modification of the method of Porterfield and Allison [6]. Cells were infected with 10-fold dilutions of virus in Hanks solution, in a volume of 0.2 mℓ. Adsorption was performed at 37°C for 1.5 h. Virus-containing liquid was removed, the cells were washed in Hanks solution and covered with agar containing the following ingredients (per 100 mℓ): Gey's A and B solutions [6] 10 and 5 mℓ, respectively, lactalbumin hydrolysate 5 mℓ, embryonic serum 5 mℓ, 2.25% soda 5 mℓ, gentamycin 20 mg, double distilled water 20 mℓ, 2% agar (Difco) 50 mℓ. The vials were incubated at 37°C for 3 days, then 0.1% neutral red (Difco) solution was added, 0.2 mℓ/vial. The vials were additionally incubated for 1 day, after which the plaques were counted. We used 3-4 vials for each dilution of virus. To determine the plaque size, we measured the diameters of 30-40 plaques by means of an ocular with a linear scale of graduations on it (0.1 mm scale factor).

Table 1. Neutralization of infectious activity of Lassa virus by homologous and heterologous sera

Serum	Serum titer in CFT with homologous antigen	Lassa virus titer after incubation with immune serum, PPU/mℓ·10^4	Neutralization index*
To Lassa virus	1:16	0.15	1.49
To lymphocytic choriomeningitis virus	1:128	4.3	0

*Index of neutralization = $\log \frac{A}{B}$, where A is titer of virus incubated with normal serum and B is titer of virus incubated with immune serum.

Neutralization reaction: To a standard dose of virus we added immune guinea pig serum to an end dilution of 1:5. The mixture was incubated in a volume of 0.2 m for 30 min at 37°C, after which titer of virus was determined.

Temperature, adsorption time, pH: To test the effect of temperature on effectiveness of plaque production, adsorption and subsequent incubation of cells were performed at the corresponding temperatures. In the study of adsorption time as a factor affecting plaque production, the cells were infected with the virus at 4 or 37°C and incubated at the appropriate temperature. Virus-containing fluid was removed every 30 min, the cells were washed and covered with agar. To test the effect of pH on adsorption, viral dilutions were prepared in 0.15 M NaCl, tris-buffered to different pH values.

Polycations, DMSO, trypsin: Solutions of DEAE-dextran (Pharmacia Firm, Sweden), protamine sulfate (Serva Firm, FRG) and trypsin (Difco, United States) were prepared in deionized water and sterilized by filtration. We also used DMSO and heparin of the Sigma Company, United States. These agents were added in various concentrations during the adsorption period or applied to the agar cover.

Results

Lassa virus-induced plaque production on Vero cells: The method of Porterfield and Allison [6] was adapted for titration of Lassa virus. Unlike the original method, we used Vero cells cultivated at the bottom of scintillation vials; gentamycin (200 μg/mℓ) was added to the agar cover and neutral red was applied to the agar cover 3 days after infection [5].

Plaques appeared on the day after addition of the dye. During the next day the plaques grew insignificantly in size; however, the number thereof remained unchanged. Plaque formation was a virus-specific process, since incubation of the virus with homologous immune serum caused reduction in number of plaques (Table 1). The quantity of plaques was a linear function of virus concentration (Figure 1).

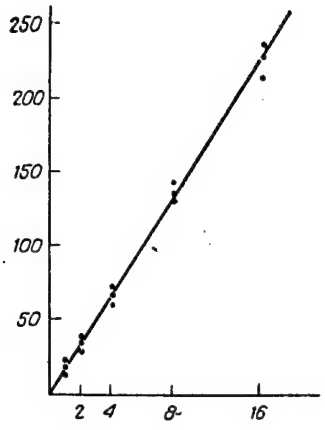

Figure 1.
Number of plaques (y-axis) formed by Lassa virus as a function of relative concentration of virus (x-axis)

The method used for titration yielded well-reproducible results. Thus, in 6 repeated titrations of the same material, the arithmetic mean of the titers constituted $1.72 \cdot 10^6$ and the standard deviation was 0.34.

A base pool of virus with a titer in excess of 10^6 PPU/mℓ, which was used for subsequent work, was obtained by cloning the virus by the above-described method. The plaques formed by cloned virus were more homogeneous in size. Thus, the diameter of plaques produced by the initial virus ranged from 0.2 to 0.8 mm. The cloned virus produced plaques with diameters of 0.3-0.5 mm (Table 2).

The virus formed plaques with equal effectiveness in the temperature range of 35 to 41°C (Table 3).

Table 2. Effects of some chemical factors on effectiveness of Lassa virus plaque production in Vero cells

Factor	Conditions of adding factor	Titer, PPU/mℓ·10^5	Plaque size, мм·10^{-1}	P
DEAE-dextran, μg/mℓ				
0	-	2,0	5,71±0,18	
10	A	1,8	5,05±0,18	>0,05
100	A	1,9	4,21±0,24	>0,05
10	C	1,1	5,19±0,23	<0,05
100	C	0,97	4,76±0,16	>0,05
DMSO, %				
0	-	1,2	5,07±0,22	
0,1%	A	2,5	3,56±0,20	>0,05
1,0%	A	1,6	4,15±0,20	>0,05
0,1%	C	1,8	3,41±0,23	>0,05
1,0%	C	1,6	3,84±0,17	>0,05
Trypsin, μg/mℓ				
0	-	1,9	5,41±0,24	
10	A	0,77	4,91±0,17	<0,05
100	A	1,5	5,69±0,21	<0,05
10	C	1,9	5,33±0,19	<0,05
100	C	1,6	5,29±0,13	<0,05

Key: A) factor added during adsorption
C) as part of agar cover
-) no factor added, control samples

Effects of physical and chemical factors on Lassa virus adsorption on Vero cells: Vero cells were infected with 10-fold dilutions of Lassa virus and incubated at 37 or 4°C. Several vials were covered with agar every 30 min and incubated further at 37°C. Viral titer was determined after addition of dye. Over 50% of Lassa virus was sorbed on Vero cells within the

first 30 min at 37°C. Optimum adsorption time was 1.5-2 h (Figure 2a). The dynamics of adsorption at 4°C were similar: 30% of the virus was sorbed in the first 30 min and maximum sorption of the virus occurred by the 2d h.

Table 3.
Effectiveness of plaque production by Lassa virus in Vero cells as a function of temperature

Temp., °C	Virus titer PPU/mℓ•10^5	Virus titer at 41°C to titer at 35, 37, 39, 41°C respectively
35	4,8	1,88
37	9,5	0,95
39	10,0	0,91
41	9,0	1,0

The adsorption curves with different pH values are illustrated in Figure 2b. Maximum adsorption was observed at pH of 8.0.

In the next experiments, we tested the effects of some chemical factors, which were added during adsorption, on effectiveness of plaque production. Adsorption of Lassa virus in the presence of DEAE-dextran did not enhance plaque production (see Table 2). On the contrary, there was a reduction of plaque size. Analogous results were obtained with protamine sulfate. Addition of DMSO or trypsin during adsorption also failed to increase the viral titer. In the case of DMSO there was a reduction of plaque size.

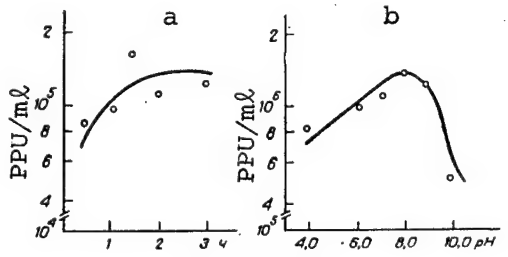

Figure 2.
Effects of time (a) and pH (b) on adsorption of Lassa virus on Vero cells

Effects of chemical factors in agar cover on plaque production: Addition of DEAE-dextran (see Table 2) and protamine sulfate (not shown) to the agar cover did not increase the titer of Lassa virus. A reduction in plaque size was observed with high concentration of polycations.

DMSO, like the polycations, had no effect on viral titer and reduced plaque size (see Table 2). Addition to the agar cover of DMSO (1.0%) together with DEAE-dextran (100 μg/mℓ) or heparin (10 μg/mℓ) did not enhance plaque production.

Addition of trypsin to agar containing serum, as well as to the cover without serum did not increase plaque production, as compared to the corresponding control samples.

Discussion

The method of Porterfield and Allison [6] with insignificant modifications was found to be adequate for titration of infectious activity of Lassa virus. The linear relationship between quantity of plaques demonstrable by this method and concentration of virus implies that one plaque is produced by one infectious viral particle.

The plaque method used enabled us to clone Lassa virus and obtain an initial viral pool in a titer of more than 10^6 PPU/mℓ.

The optimum time for adsorption of Lassa virus on Vero cells constituted 1.5-2 h. Maximum adsorption was observed at pH 8.0. Analogous findings were made with regard to Machupo, Junin, Pichinde and Tacaribe viruses [2, 4, 7, 8]. Lassa virus produced plaques with equal effectiveness at 35-41°C.

The data on the influence of DEAE-dextran on effectiveness of adenoviral plaque production in Vero cells are contradictory. There have been reports of increased effectiveness of Pichinde virus adsorption in the presence of this agent [7]. At the same time, addition of DEAE-dextran to the agar cover did not affect the number of plaques produced by Pichinde, Machupo and Parana viruses [4, 7, 9]. According to our data, DEAE-dextran and protamine sulfate did not affect the quantity of plaques produced by Lassa virus on Vero cells.

With regard to some viruses of the Phlebotomus fever group (Bunyaviridae), it has been shown that DMSO and particularly DMSO combined with DEAE-dextran or heparin had a stimulating effect on the number and size of plaques formed in Vero cells [10]. In our experiments, addition of DMSO to the agar cover during adsorption did not increase the titer of Lassa virus. There was a reduction of plaque size in the presence of DMSO. Addition to the agar cover of DMSO with DEAE-dextran or DMSO with heparin also failed to elicit an effect.

Addition of trypsin during adsorption, incubation of virus-containing fluid with trypsin and addition of trypsin to the agar cover failed to increase the titer of Lassa virus. In this regard, it must be noted that treatment of purified Pichinde virus with trypsin did not alter the electrophoretic profile of virion proteins [11].

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SOME APPROACHES TO STUDY OF EXPRESSION OF CLONED GENES

Moscow VOPROSY VIRUSOLOGII in Russian No 1, Jan-Feb 82 pp 102-103

[Editorial]

[Text] In recent years, methods of gene engineering, in particular, cloning of individual genes, both prokaryotes and eukaryotes, have gained wide use in the study of genome structure and function. This technology is based on in vitro production of recombinant DNA with the sequences of the vector and gene of interest to researchers. At the present time, the problem of creating recombinant DNA and obtaining preparative quantities of eukaryote genes in a bacterial cell can be considered essentially solved. However, producing recombinant DNA does not constitute the main purpose of work in the area of gene engineering. Expression of cloned genes is a more complex and interesting task. One can study expression of recombinant DNA in both a prokaryote and eukaryote cell, depending on the chosen vector. Synthesis of biologically active proteins (enzymes, viral antigens or individual protective determinants) in the bacterial cell is a method of developing vaccines and drugs. There could be broader application of expression of recombinant DNA in cells of higher organisms, since this is an approach to gene therapy.

At the present time, many researchers are working on the problem of obtaining eukaryote gene expression in bacterial cells. For expression of a heterologous gene, it must be under the control of a strong promoter and have the proper reading "frame" [?]. A foreign gene may carry its own promoter, with which bacterial polymerase is bound, or else it may use the promoter of vector DNA or, finally, an artificially inserted promoter. In the last two cases, it is important to select the proper reading frame. For this purpose, one can use addition or hydrolysis of a "sticky" end, or synthetic oligonucleotides in designing recombinant DNA. In recent years, the phenomenon of splicing of mRNA of higher organisms was discovered.* This transcription feature is a serious obstacle to synthesis of functionally active polypeptides in the bacterial cell since, in the first place, the intron often contains terminal codons and, in

*This phenomenon is related to the fact that the translated gene segments could be separated. For this reason, the primary gene transcript is subject to processing, as a result of which noncoding sequences (introns) are eliminated, while the rest of the molecule (exons) is reunited with production of mRNA.

the second place, with splicing there may be change in the reading frame in the middle of the mRNA molecule. A method of cloning a DNA copy (cDNA), obtained from reverse transcription of mature mRNA, was developed to overcome this obstacle.

Selection of a eukaryotic system is an independent task in the study of expression of cloned genes. In this respect, we believe that the oocytes of the Xenopus laevis African spur-toed frog are quite promising. It was shown that there is not only transcription of the injected genome, but processing and splicing of RNA in oocytes, which is of particular interest to analysis of eukaryote genes. Mature mRNA in this system is translated into functionally active protein. In addition, there is replication of the foreign genome in oocytes.

Saccharomyces cerevisiae yeast is another system that merits the closest study, since expression of some recombinant DNA has already been obtained in it.

Introduction of foreign genes into animal cells occupies a special place among experiments dealing with the study of expression of eukaryote genes. Such studies began to develop very recently. In a number of experiments, cells deficient in the thymidine kinase (TK) gene were used. It was demonstrated in several independent works that, with cotransformation of TK^- cells by the TK gene in mixture with heterologous DNA in the genome of cells that acquired the TK^+ phenotype, along with the TK gene it is possible to demonstrate sequencies that are homologous to the added foreign DNA. Lines of cells were produced, in which expression of another integrated DNA fragment is observed, along with expression of the TK gene, and in particular expression of the HB_sA_g gene of hepatitis virus B was obtained. The phenomenon of cell transformation could serve as another selective marker in analogous experiments. Thus, with cotransformation of rodent cells by DNA of adenoviruses and plasmids, sequences of both the virus and plasmid were found in the genome of transformed cells.

The genome of SV40 virus, particularly its early portion containing a strong promoter, is a convenient vector for eukaryote cells. Expression of rabbit globin genes and antigens of hepatitis virus B has already been obtained under the control of this promoter.

Thus, the foregoing indicates that several basic approaches have already emerged to obtain expression of cloned genes. However, this problem is still far from being definitively solved and each specific instance requires a special approach.

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WESTERN AND EASTERN TICK-BORNE ENCEPHALITIS IN EURASIA

Moscow VOPROSY VIRUSOLOGII in Russian No 1, Jan-Feb 82 (manuscript received 13 Mar 81) pp 103-106

[Article by V. I. Votyakov, I. I. Protas and V. M. Zhdanov, Belorussian Scientific Research Institute of Epidemiology and Microbiology, Minsk; Institute of Virology imeni D. I. Ivanovskiy, USSR Academy of Medical Sciences, Moscow]

[Text] L. A. Zil'ber et al. demonstrated that pathogenicity to monkeys and sheep of tick-born encephalitis (TBE) viruses isolated from tickes in Belorussian and the Far East is not the same [1-3]. The problem that then arose of nosological correlations between TBE in the eastern and western parts of our country became the subject of scientific discussion [4-6]. Analysis of ecological and evolutionary conceptions of the problem served as grounds for assuming that there are two species (branches) of TBE viruses [7].

In subsequent years, it was determined that one can make an antigenic differentiation between western and eastern TBE viruses with different modifications of serological tests [8-12].

In analyzing these data, we arrived at the conclusion that western and eastern TBE viruses have at least two antigens: one a group antigen and the other a species-specific one. In view of the extensive accumulation of mature virus in animals infected by the eastern strain, antibodies produced in response to group antigen are adsorbed on it. At the early stage of immunization, antibodies corresponding to the specific antigen are predominantly demonstrated in the serum of these animals. In view of lesser accumulation of virus in the organism, there is no significant adsorption of group antibodies in animals infected with the western strain, and both group and specific antibodies circulate in their blood. Consequently, at the early stage of immunogenesis in the case of infection by the western strain, two types of antibodies are produced in the animals' blood--group and specific; with infection by the eastern strain there is production essentially of one type--species-specific. For this reason, one can differentiate between western and eastern viral strains using early sera to the eastern variant, whereas this cannot be done with serum to the western variant. Evidently, the lighter fractions contain more species-specific antigens, while group antigens are inherent in heavier viral fractions. This explains the better differentiation between western and eastern viral strains with the agglutination inhibition and RDP [gel diffusion precipitin?] reactions, as compared to the neutralization reaction. However, group and type antigens are related to different structures of the compared

viruses and each time are found in random proportions. This must be taken into consideration when preparing antigens to immunize animals, obtain adsorbed sera and for neutralization reactions. For this reason, use of the above reactions of antigens of complex composition and corresponding antisera made it difficult to obtain clearcut quantitative data for differentiation between such closely related viruses as TBE viruses.

The differences in neurovirulence of the TBE complex viruses were demonstrated in experimental studies on monkeys [13]. Qualitative differences in symptomatology and course of experimental infection were observed after intracerebral infection of monkeys with Belorussian strain BR 3/3. It was established that, in spite of intracerebral injection, the virus was isolated from blood up to the 7th day, and it is only later (9th-14th postinfection days) that it accumulated in the CNS [central nervous system]. This coincided with the time of development of neurological disturbances in the monkeys, which arose after regression of general infectious manifestations, in the absence of virus from blood [14].

There were distinctly two demonstrable phases in the acute period with intracerebral infection of sheep also, which served as grounds for distinguishing the febrile-meningeal and encephalitic phases of experimental infection. About 13% of the animals expired. The eastern strain elicits almost monolithic symptomatology of encephalomyelitis with the syndrome of decerebration rigidity and death of all animals [16].

Using methods of quantitative records of degree of involvement of the main morphological elements of CNS in sheep infected with the eastern virus, we concluded that there were predominantly mesenchymal-infiltrative (neurogliotropism) and negligible degenerative lesions of ganglion cells. These findings are consistent with the above-mentioned results of neurological examination of the animals. In essence, we observed in sheep (in 91.7%) the general infectious or diffuse meningoencephalitic form of disease. There was prevalence of neuron degeneration (neuronotropism) in sheep infected with the eastern virus. Neuronophagia and glial nodules were demonstrable in over half (56.1%) the fields of vision. Such lesions were catastrophic to the organism; there was development of comatose encephalomyelitis, and the animals died (Table 1).

These data indicate that there is distinct differentiation of western and eastern TBE viruses according to a number of markers. The question of how these differences should be assessed remains debatable: are they variants (subtypes) of the same species or grounds for distinguishing independent species?

Before answering this question, let us consider some clinical aspects of the problem. Studies of the symptomatology of western TBE (over 800 cases confirmed by laboratory tests) revealed that the cardinal clinical pathogenetic feature of this neuroinfection is a two-phase acute period, which is unrelated to the type (one or two waves) of febrile reaction. At the first phase, one observes general infectious manifestations, without involvement of the nervous system in the process. The second phase does not develop before the 5th day of sickness and lasts 7-15 days. The most prominent clinical sign is serous meningitis, less often diffuse meningoencephalitis, without focal encephalitic

symptoms. About 5-6% of the patients develop paresis of the extremities, which form the syndrome of circumscribed myeloradiculoneuritis. Almost 99% of the patients recovered completely, and limited monoparesis persisted in only 0.7% of the cases. No progressive process or lethal outcome were observed.

Table 1. Main parameters of experimental infection in sheep infected with western and eastern TBE viruses

Parameters	Virus	
	western (strain BR 3/3)	eastern (strain No 198)
Acute period	Two phases	One phase
Typical clinical signs	Diffuse meningoencephalitis in 2d phase	Comatose encephalomyelitis first few days
Death rate, %	12.5	100
Pathomorphological changes in CNS	Predominant damage to all types of neuroglia	Predominant degeneration of neurons
Difference between maximum virus content in blood and CNS, log LD_{50}	0.3	2.0

A comparison of the above data to the symptomatology of eastern TBE shows the qualitative differences between the two diseases. Thus, comatose impairment of consciousness, generalized hemiplegic seizures, poliomyelitic paresis of the extremities, as well as bulbar disorders, which are inherent in eastern TBE, were not present with western TBE. The absence of any neurological symptoms in about 20% of the cases of western TBE served as grounds to distinguish its general infectious form. Some researchers draw parallels between it and the diffuse form of eastern TBE. However, the latter was characterized by transient fever (4.4±0.5 days) and transient encephalitic symptoms (paresis of cranial nerves, nystagmus, oral automatism).

The qualitative differences between the main clinical forms of western and eastern TBE precluded comparison of incidence thereof. This could be done on the basis of a concrete study of the symptomatology of western and eastern TBE [16], but without generalizing data on morbidity in different geographic regions [17]. Using such a differentiated approach, we compared our observations of western TBE patients infected via the transmissive route in Belorussia in 1952-1979 to observations made by V. I. Aleksandrov et al. [18] in Khabarovskiy Kray in 1963-1977. For statistical processing the specific share of forms in percentage was converted into G angles, in radians, using formula (3,41) and for calculation of the t criterion of difference between compared G angles we used formula (4,26) from the article by V. Yu. Urbakh [19].

As can be seen in Table 2, the share of typical forms of eastern TBE in the western sample equals zero. At the same time, the incidence of meningeal and myeloradiculoneuritis forms was higher in western TBE ($P<10^{-2}$; $P<10^{-5}$).

Our studies of the patterns of formation of viral population, biocenological, zooparasitological and other features of endemic sites convinced us of the

need for population genetics analysis in order to understand the evolution of pathogens of western and eastern TBE. We did not consider the type of virus simply as the sum of independent individuals, but as the aggregate of several genetic populations (demes) in complex relations with one another. Proceeding from the most stable genetic sign of TBE viruses--neurovirulence [20]--in the light of the clinical distinctions of the disease in humans, we isolated six populations of TBE virus in Eurasia [16]. The most distinct differences were noted between the western group (Kumlinge, Khipr, No 256, Absettarov), on the one hand, and eastern (Sof'in and Vasil'chenko viruses), on the other. Within each group there were only quantitative differences, primarily with regard to clinical manifestations in man. In singling out the viral populations from the standpoint of human symptomatology of the disease, we proceeded from the thesis that one species--man--is the indicator of genetic distinctions of the populations.

Table 2. Incidence of clinical forms of eastern and western TBE

Form	Khabarovskiy Kray*		BSSR		P
	abs.	%	abs.	%	
Diffuse (encephalitic)	251	30,9	0	0	10^{-5}
Systemic infectious	0	0	46	15,6	10^{-5}
Meningeal	222	27,3	226	76,6	10^{-5}
Focal meningoencephalitic	196	24,1	0	0	10^{-5}
Poliencephalomyelitic	116	14,3	0	0	10^{-5}
Myeloradiculoneuritic	28	3,4	23	7,8	10^{-2}
Totals . . .	813	100	295	100	

*Data of V. I. Aleksandrov et al. [18].

The different viral populations in the west and Far East are separated by almost 10,000 km. If we were to consider all populations to refer to one viral species, the European part of the USSR should be considered the center of its range with a more stable gene pool. However, two different viral populations are recorded there, one of which is similar to the western TBE virus (two-wave meningoencephalitis) and the other, to the eastern (Ural-Siberian variant). As we move away from the zone where western and eastern populations adjoin, we find that they are more homogeneous. Consequently, there are two centers in the range--western and eastern. From the standpoint of population genetics, this is indicative of existence of two types of viruses. Using the method of oligopeptide mapping in comparative studies of different strains of western and eastern TBE viruses, as well as Scottish encephalitis (SEO) virus, Langat and others, it was demonstrated that all TBE viruses have a similar shell [21]. However, in spite of some differences between one another, the western strains differed from eastern ones. Only the Absettarov strain, which is a representative of the population in the northwestern part of European USSR, presented some resemblance to the Sof'in strain. At the same time, Khipr and No 256 strains, and even SEO virus did not present such a similarity, which is consistent with the data of Clarke [12] concerning the antigenic composition of viruses in eastern Europe and western parts of the USSR. All this confirms the existence of two

genetic groups of viral populations, western and eastern. Viruses with both western and eastern features can be found on the borderlines between them. Western TBE virus is an independent species that lives in its inherent ecosystem (I. ricinus with the corresponding vertebrate fauna), and this ecosystem has left its imprint on the antigenic structure of the virus. This species inhabits Europe and adjoins the other species, eastern TBE virus (ecosystem of I. persulcatus with the corresponding vertebrate fauna) in the northwestern and eastern parts of European USSR. It can be assumed that the viruses had a common ancestor. Their separation occurred as a result of geographic isolation at first, then existence in different ecosystems and, finally, genetic isolation [16].

Thus, the aggregate of specific differences between pathogens, qualitative differences in symptomatology and pathogenesis of neurological manifestations of disease, along with the results of population genetics analysis, are indicative of nosological differences between eastern and western TBE. For this reason, it is high time to amend the International Classification of Viruses. Strain No 256 and strain BR 3/3, which were first isolated in Belorussia by M. P. Chumakov et al. [22] from ticks and V. I. Votyakov et al. [16] from sick humans, should be added among TBE viruses. In addition, all of the western viruses should be combined in one species, Occidentalis encephalitis virus [7]. Moreover, western TBE, which was first described in the USSR (Belorussia), should take the place of central European TBE in the International Classification of Diseases [23].

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FIFTH INTERNATIONAL VIROLOGICAL CONGRESS [STRASBOURG, FRANCE, 1981]

Moscow VOPROSY VIRUSOLOGII in Russian No 1, Jan-Feb 82 pp 109-110

[Article by B. F. Semenov and S. G. Drozdov (Moscow]

[Excerpts] Hemorrhagic Fevers

The results of studies pursued in recent years of diseases of diverse etiology and from different geographic regions were represented in the proceedings of the working conference and papers accompanied by displays.

HALSTEAD (United States) delivered a paper dealing with dengue fever and its rising significance. In the last 25 years, there were more than 500,000 recorded cases of the hemorrhagic form of dengue, 14,000 deaths, and this disease is recorded in 62 countries with a total population of 1.7 billion. The mechanism of development of the most serious form of dengue with hemorrhagic symptoms, associated with the shock syndrome has not yet been determined. The author upholds the hypothesis that interaction on cell membranes plays the leading part in development of this syndrome. In 1980, serological and virological studies were begun in Thailand of the cases of hemorrhagic dengue for the purpose of verifying this hypothesis and they are scheduled for 5 years.

Diverse data were submitted by KILEY et al. (United States) on morphological, chemical, genetic and antigenic features of Marburg and Ebola viruses, which are indicative of their great similarity. The authors propose that these viruses be isolated in a separate family, Filoviridae (from the Latin, filum--filament, which reflects the filamented form of the virion).

WEISSENBACHER (Argentina), who is actively developing research on Argentine arenaviral hemorrhagic fever, reported demonstration in experiments on C. jacchus marmosets of the capacity of Tacaribe virus to produce immunity to subsequent infection by Junin virus, the pathogen of Argentine hemorrhagic fever.

McCORMICK et al. (United States) reported the results of many years of research on Lassa fever in Sierra Leone. They defined the clinical signs of this disease, demonstrated the infection of women's milk, urine, pharyngeal and conjunctival washings, pleural and cerebrospinal fluid in severe cases associated with hemorrhages; they found cases of subclinical infection in humans and obtained

data which may be indicative of the role of Muridae [or Myomorpha] rodents in the spread of the viruses in sites.

A series of papers was concerned with hemorrhagic fever with the renal syndrome (HFRS, or epidemic neuropathy, hemorrhagic nephrosonephritis, Korean hemorrhagic fever). Etiological studies of this disease and attempts to cultivate the pathogen virus are being made with particular intensity in recent times, after LEE (1978) discovered specific viral antigen in the lungs of field mice (Apodemus agrarius) trapped in sites of the disease and demonstrated that it is possible to pass the viral agent on these animals. At the congress, LEE et al. (South Korea, United States) submitted data on the physicochemical properties of the viral pathogen of HFRS submitted to passages on field mice. According to the data of these authors, the virus contains RNA, passes through filters with 100 nm pores but is retained by filters with 50 nm mesh; it is inactivated by lipoid solvents and ultraviolet light, as well as ordinary disinfectants. The authors studied the distinctions of infection in infected field mice, which was characterized by a tendency to change into a chronic form (isolation of virus from urine in some animals for 1 year after infection).

S. G. DROZDOV (USSR) submitted the data of a team of researchers (Ye. A. Tkachenko, A. P. Ivanov, G. V. Rezapkin, M. A. Donets, T. K. Dzagurova, S. G. Drozdov) on use of immunosorbent methods (radioimmunological--RIA--and immunoenzymatic--ELISA) to detect hemorrhagic fevers caused by arenaviruses (Junin, Machupo, Lassa), CHF [Congo hemorrhagic fever] group and HFRS viruses. The immunosorbent methods were found to be highly sensitive and specific. Use thereof made it possible to rapidly determine the etiology of HFRS in several sites of the USSR, as well as to demonstrate some of the distinctions of infection and immunity in humans and to obtain new data on ecology of the virus.

The paper of AL-MOSLIH et al. (Iraq) was concerned with an outbreak of hemorrhagic fever in Iraq in 1979. The authors isolated from patients' blood a virus referable to the CHF-Congo group, and it was used to conduct seroepidemiological studies.

Safety Problems in Virological Laboratories

The paper of McCORMICK et al. (United States) contained information about equipment and medical procedures that assure safety when working with hazardous viruses (Lassa, Marburg, Ebola) in a field laboratory (Sierra Leone) and special laboratory with maximum level of biological safety (Atlanta, United States).

KALTER (United States) made a comprehensive survey of problems of biological hazard when working with primates, as well as possibilities and methods of eliminating or lowering it. This problem is of exceptional importance, since primates are capable of transmitting and spreading a significant amount of highly pathogenic viruses, in particular, B virus (herpes virus simiae) and Marburg virus, which cause fatal diseases in man.

JOHNSON (United States), DOWDLE (United States), FABIYI (Nigeria) and McCORMICK (United States) participated in the brief discussion of the papers.

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ECHOLOCATION PROCESS AMONG HORSESHOE BATS AFTER PARTIAL AND TOTAL ABLATION OF INFERIOR COLLICULUS

Leningrad VESTNIK LENINGRADSKOGO UNIVERSITETA: BIOLOGIYA in Russian
No 15, Issue 3, Aug 81 (manuscript received 12 May 80) pp 74-81

KONSTANTINOV, A. I., MOVCHAN, Ye. V. and ZHARSKAYA, V. D.

[Abstract] The goal of the present work was to study the functions of various segments of inferior colliculus of horseshoe bats in respect to the echolocating process. Specifically, functional disorders of the specialized locating system were investigated after partial or total ablation of the inferior colliculus: hearing sensitivity, range of target detection and compensation of the Doppler effect. Unilateral ablation of the inferior colliculus had no effect on the spacial orientation of experimental animals, except that in the early phases the range orientation was affected. Bilateral ablation of the lateral cortex or total exclusion of one of the inferior colliculi and the lateral cortex of the other, as well as bilateral removal of caudal segments showed no effect on the flight behavior or on performance of the echolocating system. Bilateral ablation of dorsolateral segments and coagulation of caudal and dorsal segments had also no marked effect on the bats. Only bilateral ablation of more than 50% of the nucleus led to considerable instability of the resting frequency preventing the bats from utilizing the Doppler effect for orientation. It was concluded that the disorders in the echolocating system are connected with disorders of the afferent connections of the inferior colliculus with frontal colliculus and reticular formations. Figures 4; references 16: 10 Russian, 6 Western.
[125-7813]

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THEORETICAL AND PRACTICAL ASPECTS OF USING ACOUSTIC REPELLENTS TO SCARE BIRDS, PART 1: INTERSPECIFICITY AND GEOGRAPHIC (REGIONAL) DISTINCTIONS OF ACOUSTIC REPELLENTS

Moscow BIOLOGICHESKIYE NAUKI in Russian No 1, Jan 82 (manuscript received 19 Dec 80) pp 45-49

[Article by A. V. Tikhonov and V. S. Shevyakov, recommended by the Department of Vertebrate Zoology, Moscow State University imeni M. V. Lomonosov]

[Text] A study was made of interspecificity of alarm and distress signal effects on Larines, Corvidae and starlings, as well as geographic aspects of using these signals as acoustic repellents. It was established that repellent reactions are induced in birds by signals not only of closely related species, but of systematically distant groups that are biocenotically close neighbors. The interspecificity of repellent signals is manifested the most during the nesting period.

The problem of controlling bird behavior covers a wide range of questions of concern to aviation, agriculture, hunting, environmental protection, etc. [1, 2]. Acoustic repellents are prominent in the general problem of controlling the behavior of birds. They are used to scare off birds from the territory of airports, agricultural products, fisheries and others.

The reactions of birds to alarm and distress signals is a defense reaction, which developed in the course of evolution and preserves the integrity of a population [5]. As a rule, colonial birds or those that form flocks are scared off to protect objects of economic importance. The defense reactions (scattering, flying away) of these groups present adaptational distinctions [5]. It is important to analyze such adaptive features of defense reactions as interspecificity of effects of alarm and distress signals, as well as geographic aspects of using them as acoustic repellents, in order to continue development of methods of controlling bird behavior.

In 1976-1980, we conducted studies of acoustic communication and behavior of birds in nesting colonies and flocks; we conducted experiments with the use of acoustic repellents to scare birds away from economically important objects in Moscow, Odesskaya, Khersonskaya, Kalininskaya, Astrakhanskaya and Murmanskaya Oblasts, Lithuanian SSR, Chukotsk National Okrug and on Kamchatka Peninsula. The methodological procedures and distinctions of translating acoustic repellents were described previously [5, 7].

Interspecificity of Effects of Acoustic Repellents

Various species of birds are found in the areas of airports, granaries and other agricultural objects. Mixed flocks of Corvidae consist of rooks, hooded crows and jackdaws; the Larines flocks include black-headed, common and herring gulls, various species of terns. In many cases, starlings and rock-doves are present along with Larines and Corvidae. We devoted special attention to the following questions in order to determine the distinctions of effects of acoustic repellents on birds of different systematic groups: 1) nature and efficacy of alarm and distress signals in mixed flocks of closely related species; 2) distinctions of reactions to alarm and distress signals of birds belonging to systematically distant groups.

Corvidae: In the fall and spring, alarm and distress signals of any bird species contained in the flock have a repellent effect on mixed flocks. In view of the distinctions of flock reactions, there is no purpose to comparing the effectiveness of the signals used in this case. Flight and involvement in the reaction of other species of birds could be based on elements of imitation in the flock.

The reactions of mixed flocks to repellent signals of systematically remote groups are of much greater interest. The alarm and distress signals of the herring-gull, arctic tern and starling have different repellent effects on Corvidae. The "latency" period of the reaction is shortest in translating distress signals of the starling. In this case, the phase of circling rapidly changes to the scattering phase. Translation of repellent signals of gulls elicits only flight and landing of Corvidae.

Larines: As was the case for Corvidae, group patterns of behavioral reactions are observed in mixed flocks of gulls. In our experiments, we used situations which precluded the factor of group involvement in the reaction. As can be seen in the Table, the distress signals of terns induce a repellent reaction in gulls. The repellent reaction, including all of its stages [5], ended with landing of birds in nesting colonies, whereas in feeding places it ended with scattering for a short time of some of the birds. The repellent signals of birds of systematically remote groups elicited typical defense behavior in gulls only when in nesting colonies. When these repellents were used for migrant flocks their efficacy was low.

The "latency" period of the reaction was of minimal duration when translating alarm and distress signals of birds in closely related groups, and it increased with the use of signals of birds of systematically remote species. The change in duration of total reaction time presented the opposite pattern (see Table).

Starlings: Model experiments conducted in starling nesting places revealed that repellent reactions are induced in them only by signals of certain bird species. Alarm and distress signals of Corvidae and house sparrow elicited a strong defense reaction, while the repellent signals of gulls and terns were less effective.

Distinctions in reactions of herring, black-headed and common gulls to translation of different variants of acoustic repellents

	Birds' relationship to territory	Reaction latency period, s	Distinctions of group behavior	Reaction period, s	Flew away, % of initial number
			Herring gulls		
Distress signal of common tern	Nesting colony	2	Flight-approach-circling-alighting	340	--
	Feeding place	2-4	Flight-approach-circling-scattering	290	40
Distress signal of arctic tern	Nesting colony	-	Same	300	--
	Feeding place	1-2	"	240	60
Distress signal of common gull	Nesting colony	-	"	360	10
	Feeding place	-	"	300	100
Distress signal of rook	Nesting colony	3-5	"	280	--
	Feeding place	3-7	Flight-circling-scattering(alighting)	250	20
Distress signal of starling	Nesting colony	3-4	Flight-alighting	200	--
	Feeding place	7-9	Same	240	--
			Black-headed and common gulls		
Distress signal of arctic tern	Black-headed gull:				
	nesting colony	1-2	Flight-approach-circling-alighting	320	--
	feeding place	3-5	Flight-approach-circling-scattering	270	80
	Common gull: fall migratory flock	2-4	Flight-circling-scattering	190	70
Distress signal of herring-gull	Black-headed gull:				
	nesting colony	-	Flight-approach-circling-alighting	380	--
	feeding place	1-2	Flight-approach-circling-scattering	300	100
Distress signal of rook	Black-headed gull:				
	nesting colony	6-9	Flight-circling-alighting	220	--
	feeding place	3-5	Flight-circling-scattering(alighting)	190	30
	Common gull: fall migratory flock	2-3	Flight-scattering (alighting)	160	40

In the fall, starlings in flocks including Corvidae were readily scared off by repellent signals of any of the species in the mixed flock [6]. The efficacy of different variants of acoustic repellents varied for different flocks of crows. Corvidae signals have a good repellent effect, and the signals of the gull family are less effective.

A reciprocal effect of alarm and distress signals was often observed among Pelecaniforms [or Steganopodes] and Ciconiiformes birds [5]. The distress signals of the common heron affect all Ciconiiformes species and cormorants. The repellent signals of cormorants elicit s trong defense reactions in Ciconiiformes.

Geographic (Regional) Aspects of Using Acoustic Repellents

The efficacy of acoustic repellents in some geographic regions and unstable effects thereof in others are often related to sensitivity of the local population to alarm and distress signals of birds from another part of the range [4, 8-10]. In order to check the regional distinctions of acoustic repellents, we conducted a series of experiments on birds of different systematic groups. The set-up of model experiments included recording the alarm and distress signals of birds in one of the regions in the range of that species and subsequent translation of these records for a population from another region.

The results of these experiments revealed that alarm and distress signals of black-headed gulls of the Rybinskiy Reservoir (Kalininskaya Oblast) and Odesskaya Oblast elicit a marked repellent reaction in gulls of Lithuanian SSR. Records made in Lithuanian SSR were effective in scaring off common gulls from the territory of spawning grounds of the Odessa Fish-Processing Plant. Black-headed gulls of Kamchatskaya Oblast react to records made in Lithuania, Odesskaya and Kalininskaya Oblasts. We also found that there was a reciprocal effect of signals of herring-gulls in different parts of the range (Murmanskaya Oblast--Chukotka). The behavioral distinctions of gulls in translating repellent signals of birds from other parts of the range were manifested by some increase in "latency" period of the reaction. However, these differences were quite insignificant. In some cases, the birds did not fly up to the sound source as they developed the repellent reaction: after they took off there was a circling phase followed by scattering.

Common and arctic terns do not differ in their repellent reactions to distress signals of birds in local populations and geographically distant regions. In all of the experimental variants, we found an expressive defense reaction in nesting colonies, with minimal "latency" period. All of the phases of the repellent reaction were distinctly manifest.

We also failed to demonstrate geographic "insusceptibility" among Corvidae in different parts of the range. Some increase in reaction "latency" period was observed in translating distress signals of hooded crows and rooks from Odesskaya Oblast by this species of birds in Lithuanian SSR (in the fall). At the same time, the efficacy of repellent signals of Corvidae from different parts of the range was virtually the same with regard to nesting colonies. There was no change in structure of the group repellent reaction in all of the translation variants.

The phenomenon of geographic specificity of alarm and distress signals was not demonstrated for the starling either. In both the nesting and fall periods, signals of birds from different parts of the range elicited a repellent reaction in the starling. Analogous findings were made by Kazakh ornithologists [3]. We also failed to demonstrate geographic "insusceptibility" to alarm and distress signals among Pelecaniformes and Ciconiiformes [5].

Thus, a repellent reaction is induced in birds by alarm and distress signals not only of closely related species, but birds of systematically remote groups. There is maximum manifestation of interspecific effects of repellent signals in the nesting period. Signals of birds referable to systematically distant groups have a considerable interspecific effect on their immediate neighbors-partners in the biocenosis.

The results of our studies failed to confirm the existence of the phenomenon of geographic "insusceptibility" of birds within their range to species-specific alarm and distress signals.

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FUNCTIONAL CHARACTERISTICS OF MECHANORECEPTORS OF UNCILIATED TYPE

Leningrad VESTNIK LENINGRADSKOGO UNIVERSITETA: BIOLOGIYA in Russian No 21, Issue 4, Nov 81 pp 75-80

[Article by N. P. Alekseyev]

[Text] The sense organs are used to learn about the outside world, as well as in activity of animals. Receptors are the initial element of all sense organs; they constitute highly sensitive biological sensors, by means of which there is perception and transformation into nervous signals of changes in the exogenous and endogenous environment.

Mechanoreceptors are very widespread in animal organisms, and they play an exceptionally important role in their vital functions. To date, there are several classifications of mechanoreceptors. The best can be considered the one that separates mechanoreceptors into pilar-ciliated and unciliated (U. Thurm, 1969; O. B. Il'inskiy, 1975). In pilar-ciliated mechanoreceptors, perception of a stimulus occurs by means of special cilia or pili. No special structures were found in aciliated receptors. It is believed that the act of reception occurs in them by means of the membrane of branched or unbranched endings of afferent nerve fibers. Numerous studies have shown that receptors of the unciliated type perform mechanoreceptor function in different tissues and organs, with the exception of the lateral acoustic system, of vertebrate animals. In some unciliated mechanoreceptors, the nerve element is contained in one or several capsules; however, in most cases these receptors have no special capsules, and they are united under the general name of "free" nerve endings.

It should be noted that technical difficulties arise in the study of properties of unciliated mechanoreceptors by means of recording impulsation activity. Thus, the study of mechanoreceptors of viscera and skin is made difficult by the fact that most of the nerve fibers forming nerve endings are fine unmyelinated and myelinated fibers. In addition, the integument of animals is covered with hair, with the exception of small areas. The hair follicles have well-developed sensory innervation. The marked afferent impulsation from these endings when the skin is submitted to mechanical stimulation obscures the responses of other mechanoreceptors. In addition, the hair cover makes it difficult to pinpoint and stimulate mechanoreceptors of the skin with strictly measured mechanical stimuli.

In this report, we submit data on functional characteristics of solitary mechanoreceptors of the aciliate type in the papilla and parenchyma of the mammary gland. There were several reasons for selecting mechanoreceptors of the mammary gland: 1) there is no hair on the papilla and areola, which facilitates localization of mechanoreceptor units and permits use of mechanical stimuli with highly precise changes in amplitude, duration and gradient; 2) the parenchymal mechanoreceptors together with afferent fibers can be isolated from the organ and their function submitted to pharmacological analysis; 3) depending on the stages of lactation, the mammary gland and papilla undergo appreciable morphological and functional changes, so that it is of particular interest to study the functional characteristics of the receptors at different stages of lactation.

First, let us dwell briefly on the morphological distinctions of afferent endings of the mammary papilla and parenchyma. Studies of the terminal segments of nerve fibers in the papilla and parenchyma of the mammary gland made it possible to distinguish three types of nerve endings (I. I. Grachev, N. P. Alekseyev, 1980).

The first type of nerve ending is a bulb-shaped dilatation formed by nerve fibers situated mainly in the reticular layer of the skin. There are many mitochondria with dense matrix within the endings. The proximal end contains microtubules oriented along the length of a nerve fiber. Nerve endings of this type are surrounded by collagen fibers.

Nerve endings of the second type are encountered in the papillary skin layer of the guinea pig mammary papilla, right near the basement layer. As a rule, there are 2-3 terminals in the grooves of one lemmocyte, and the lemmocyte does not cover the entire surface of the endings. Mitochondria are seen in the axoplasm of the nerve endings.

The third type of ending is encountered most often in the skin of the mammary papilla of the albino rat. There are 2-3 nerve endings per lemmocyte. Mitochondria are very rarely encountered in the axoplasm of these endings.

Only unmyelinated nerve fibers, 1.5-3 μm in diameter, were rather seldom seen in the parenchyma of the mammary gland. As the nerve endings come close to their endings, they gradually exit from the lemmocyte groove and become "bare" fibers wanting in a sheath. The terminal elements of these fibers are represented in the form of free nerve endings, which can be referred to the third type. Our morphological experiments failed to demonstrate encapsulated receptors.

To date, on the basis of electrophysiological experiments, five types of mechanoreceptors have been identified in the hirsute and hair-free skin of man and animals (see survey by A. B. Vallbo et al., 1979): 1) slowly adapting mechanoreceptors of the first type (SAI), 2) the same of the second type (SAII), 3) rapidly adapting mechanoreceptors (RA), 4) rapidly adapting receptors of the Pacini corpuscle type (PC) and 5) rapidly adapting mechanoreceptors of hair follicles. According to morphological data, it is believed that SAI correspond to Merkel corpuscles, SAII to Ruffini corpusles, RA to Meissner or Krause corpuscles, PC to Pacini and other Pacini-like corpuscles.

Electrophysiological studies revealed slowly and rapidly adapting mechanoreceptors in the papilla and parenchyma of the mammary gland (I. I. Grachev, N. P. Alekseyev, 1980).

Slowly adapting mechanoreceptors (SA) occasionally presented spontaneous impulsation at a frequency of 1-3 impulses/s at rest. Stepped mechanical stimulation of papillary and areolar SA elicited the following impulsation reaction (Figure 1 [none of cited figures is reproduced]). The increase in amplitude of mechanical stimulation was associated with increase in frequency of afferent action potentials (AP). Upon reaching a constant level, AP frequency was at a maximum and rapidly decreased to the values determined by stimulus amplitude. During constant mechanical displacement, there was slow decrease in AP frequency, one group of SA presenting regular (SAI) (Figure 1b) and the other irregular (SAII) (Figure 1a) impulsation. Upon termination of stimulation, there was a period of depression of spontaneous AP, which was directly related to amplitude and duration of mechanical stimulation. The coefficient of variation (ratio of standard deviation of interval between pulses to its mean value), which characterizes the degree of irregularity of the interpulse interval, increased from 0.3 to 1.4 in the course of slow adaptation for SAII and decreased from 0.23 to 0.12 for SAI. A comprehensive study of mean frequency of SAI and SAII as a function of amplitude of stepped [staggered, step-by-step] mechanical stimulus revealed that this function is approximated with a line, with a high coefficient of correlation ($r = 0.98$). However, such a linear function was not observed at low (less than 50 μm) and rather high (over 300 μm) amplitudes of mechanical displacement. Analysis of the experimental data revealed that the amplitude of mechanical stimulus as a function of mean AP frequency had a tendency toward forming an S-shaped curve (Figure 2a). Parenchymal SA responded to both tactile mechanical stimulation and stretching the gland. However, only the tactile mechanical stimuli could be graded rather precisely in amplitude, duration and gradient. SA impulsation reaction to a stepped tactile mechanical stimulus was analogous to the impulsation responses of slowly adaptive mechanoreceptors of the papilla and areola. At the same time, the mean AP frequency of the static part of the SA response to maximum amplitudes of stimulation did not exceed 15-20/s and dynamic surge frequency did not exceed 60-70/s, whereas in slowly adapting receptors of the papilla and areola, the mean frequency of the static part of the response could reach 50-60/s and the dynamic peak--300/s. SA of the parenchyma were of two types: SA with regular and SA with irregular impulsation activity.

One of the physiological methods of classifying receptors is based on examining impulsation under the influence of serrate ["saw-tooth"] mechanical stimuli built up at different rates. This made it possible to determine which is an adequate stimulus for a receptor--amplitude of displacement or rate of build-up of the mechanical stimulus (P. R. Burgess, E. R. Perl, 1973). The linear relationship between amplitude of displacement of the skin and frequency of afferent AP for SA indicates that they are amplitude detectors. When an amplitude detector is submitted to serrate stimuli with different rates of build-up, the immediate frequency of AP increases with increase in amplitude, and it is unrelated to the rate of build-up of the stimulus provided there is no adaptation. If adaptation processes are present, the receptor's response also depends on the rate of build-up of the stimulus. Then, at rather low rates of

stimulus build-up, AP frequency will not increase with increase in amplitude of stimulation, since the adaptation process (i.e., process, as a result of which AP frequency decreases) compensates for increase in AP frequency that occurs under the influence of increasing the amplitude of the stimulus. With increase in rate of build-up of stimulation, the influence of adaptation diminishes and AP frequency will increase with increase in stimulus amplitude. The immediate [instantaneous] frequency of both types of SA (Figure 3a) of mammary and areolar receptors of the mammary gland showed virtually no change upon stimulation with serrate mechanical stimuli, the rate of which constituted hundredths of a μm/ms, when the amplitude of serrate stimulation was increased. Moreover, with increase in build-up rate, we observed upward shift of the lines of the function "immediate AP frequency--amplitude of mechanical stimulus." Thus, the SA of the papilla and areola can be classified as mixed detectors, namely, amplitude--rate detectors. In response to serrate mechanical stimuli differing in build-up rate, the immediate frequency of AP of parenchymal SA increased linearly with increase in stimulus amplitude. A linear function was observed up to 700-750 μm. At high amplitudes of stimuli, AP frequency began to drop. It should be noted that parenchymal SA responded with an increase in AP frequency only when the rate of build-up of the serrate stimulus did not exceed 0.2-0.5 μm/ms. At faster build-up rates, there was no increase in AP frequency. These data, as well as the low index of AP frequency of the dynamic and static parts of the responses, enabled us to classify parenchymal SA as detectors of amplitude and slow rates.

Rapidly adapting mechanoreceptors (RA) of the papilla and areola of the mammary gland were of two types. At rest, both types of RA had no spontaneous impulsation and generated afferent AP only with change in the mechanical stimulus (Figure 4a, 1). Mean frequency of the first type of RA (RAI) increased linearly with increase in rate of stimulation (Figure 4a, 2-5) and the function was approximated by a straight line with high coefficient of correlation ($r = 0.99$)(Figure 2b). The second type of RA (RAII) also showed an increase in frequency with increase in rate of stimulus build-up (Figure 4b), but the "speed threshold," i.e., rate of build-up of mechanical displacement at which the receptors start to generate AP, was higher in RAII than RAI. In addition, with increase in rate of stimulus build-up, the number of afferent AP in the RAII responses did not exceed 2-3. It has already been noted that the use of serrate stimuli is an effective means of classifying receptors. Mechanoreceptors, whose impulsation activity depends on the rate of change in a mechanical stimulus and does not depend on amplitude are identified as rate detectors. For rate detectors, the immediate AP frequency as a function of amplitude of mechanical stimulation at different build-up rates will be rendered graphically as a family of lines parallel to the axis on which the stimulation amplitude is plotted (A. G. Brown, A. Iggo, 1967; P. R. Burgess, E. R. Perl, 1973). Statistical processing of the results revealed (Figure 3b) that, for RAI, the correlation between immediate AP frequency and stimulus amplitude at different build-up rates is approximated by lines that may be considered parallel to one another and the x-axis (axis of change in amplitude), since the coefficient of inclination of the lines changes from -0.09 to +0.08. Consequently, RAI can be classified as rate detectors. Studies of the effects of sinusoid mechanical stimulation revealed that the threshold of generation of RAI AP is in the range of 10-25 Hz. Upon stimulation by sinusoid mechanical

stimuli, RAII generated AP at a relatively high stimulation frequency (over 100 Hz). The RAII responses are typical of receptors of the Pacini corpuscle type, in which the lamellar capsule serves as a high-frequency filter (S. J. Hubbard, 1958). RAII were classified as impact [impulse] or acceleration detectors. Thus, there is an entire set of physiological sensors of amplitude, slow and fast rates in the papilla and parenchyma of the mammary gland.

With regard to their functional characteristics, SAI (with irregular impulsation) resemble the SAI that correspond to Merkel corpuscles according to data in the literature. SAII (with regular impulsation) are similar to the properties of SAII that correspond to Ruffini corpuscles according to the literature. RAI resemble the properties of RA while RAII resemble PC receptors. However, let us recall that our morphological experiments failed to demonstrate unciliated encapsulated receptors.

Thus, the above data lead to the important conclusion that coding of information about the parameters of a mechanical stimulus can be done not only by encapsulated, but "free" nerve endings.

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CSO: 1840/188

UDC 537.591 : 581.142

USING PHYTOCHROME-DEPENDENT REACTION IN EVALUATING EFFECTS OF SPACE FLIGHT FACTORS ON PLANT ORGANISM

Riga IZVESTIYA AKADEMII NAUK LATVIYSKOY SSR in Russian No 11, Nov 81 (manuscript received 16 Jun 81) pp 94-99

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[Abstract] The title reaction was tested on air-dried "Krupnokochannyy" lettuce seeds that were sent on "Kosmos" orbiting flights. Some of these seeds were exposed to irradiation, while others were shielded in a protective container. The seeds were evaluated for sprouting, plant growth and enzyme activity after return from orbit. Irradiation reduced sprouting by about 25%, and suppressed the phytochrome-dependent reaction, but at later growth stages deviations from control plants gradually disappeared. Under red light exposure, the Ph_{660} inactive molecule converted to the active Ph_{730}; this reaction also reversed itself. The phytochrome-dependent reaction was determined to be connected to certain changes in the cell genome. Figures 2; references 16: 7 Russian, 9 Western.
[144-12131]

UDC 579.842.11: 570.252.5]: 612.017.1

ISOLATION AND CHARACTERIZATION OF E. COLI PLASMID-DETERMINED K88 ANTIGEN

Moscow ZHURNAL MIKROBIOLOGII, EPIDEMIOLOGII I IMMUNOBIOLOGII in Russian No 11, Nov 81 (manuscript received 9 Apr 81) pp 31-36

VODOP'YANOV, S. O., ALEKSEYEVA, L. P. and MISHAN'KIN, B. N., Rostov-na-Donu Scientific Research Institute of Antiplagues

[Abstract] Pure K88 antigen was isolated from E. coli cells using two different methods in order to compare their efficiency. A study was made of K88 properties and of its suggested affinity for both biotypes of Vibrio

cholerae. The methods used to isolate K88 were differential ultracentrifugation and isolectric precipitation: the former was the better method, causing no cell damage during the process. The pure K88 antigen obtained was of a protein nature, with no carbohydrate of lipid components; it showed a single absorption peak at 280 nanometers. Its molecular weight was about 25,000 daltons, with a sedimentation coefficient of 16.6 S; this coefficient is less than half that found in other work (Stirm, Orshov et al 1967). Tests on mice showed that K88 exerted no toxic effects. Its transmission to recipient cells sharply increased adhesion to the intestinal mucosa. No antigen affinity was found between K88 and either of the two Vibrio cholerae biotypes. Figures 4; references 15: All Western.
[186-9642]

UDC 579.841.94: 579.252.4

PREPARATION AND STUDY OF BORDETELLA PARAPERTUSSIS STRAINS 17903 CARRYING Rts-1 and RP-1 PLASMIDS

Moscow ZHURNAL MIKROBIOLOGII, EPIDEMIOLOGII I IMMUNOBIOLOGII in Russian
No 11, Nov 81 (manuscript received 15 Apr 81) pp 48-50

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[Abstract] Strains of Salmonella carrying the Rts-1 plasmid with an added Tp10 transposon and E.coli strains carrying the RP-1 plasmid with an added Tp9 transposon were used as donors and B. parapertussis strain 17903 as the recipient in hybridization studies to clarify the possible use of plasmids and transposons in investigations of poorly studied microorganisms, in particular of the genus Bordetella. Both these genetic elements are known to participate as determinants in the transmission of drug resistance. Conjugative plasmid Rts-1 (Tp10) in cells of B. parapertussis imparts resistance to kanamycin and tetracyclin; plasmid Rp-1 (Tp9) imparts resistance to kanamycin and chloramphenicol. Incubation at nonpermissive temperature (41°C) leads to the formation of heat-resistant clones of Rts-1 (Tn10) 17903 (frequency 10^{-3}) which retain the markers for drug resistance but lose their ability for conjugation. References 6: 3 Russian, 3 Western.
[186-9642]

UDC 577.11 : 005

STUDY OF CITATIONS IN SOME FIELDS OF MOLECULAR BIOLOGY AND BIOORGANIC CHEMISTRY

Moscow VESTNIK MOSKOVSKOGO UNIVERSITETA, SERIYA 16: BIOLOGIYA in Russian
No 1, Jan-Mar 82 (manuscript received 14 Jan 80) pp 60-67

GUS'KOVA, L. I., LIPITSKAYA, I. Ya., BELIKOVA, M. P. and ZALESOVA, Z. G.

[Abstract] Citation frequency of works by Soviet scientists has been studied previously (Vasil'yev, 1967, Orient, 1967, Granovsky, 1974, Belikova and Zalesova, 1976); the present study considers works by collaborators at the Interdepartmental Laboratory for Problems in Molecular Biology and Bioorganic Chemistry imeni A. N. Belozerskiy. The frequency of citation of every primary co-author was checked against the Science Citation Index for 13 years in terms of total published and cited works, total number of citations, number of citations in relation to number of published works, and average number of citations per year. Results showed that an article's "active life" lasted 10 years, after which it became archival material, although some works published prior to 1967 continue to be cited on a regular basis. Topical urgency also affected citation frequency, and the place and language of publication were found to be less important than scientific significance. Some substantive areas showing highly active scholarly endeavor, based on this study of citations, were RNA-containing viruses in animals, nucleic and protein reactions in ribosomes and in some viruses, structure and enzymatic modification of the genome and membrane bioenergetics. The data can be used to assess effectiveness of scientific groups and individuals. References 9: 7 Russian, 2 English.
[140-12131]

UDC: 599.32:595.775.659

RODENT CAPACITY TO RID THEMSELVES FROM SPECIFIC AND NONSPECIFIC FLEA SPECIES

Moscow ZOOLOGICHESKIY ZHURNAL in Russian Vol 60, No 1, Jan 81 pp 165-167

[Article by N. A. Nikitina and G. A. Nikolayeva, Institute of Epidemiology and Microbiology, USSR Academy of Medical Sciences, Moscow]

[Text] Investigation of rodent capacity to brush fleas away is of theoretical and practical importance: it explains why various host species are infested with fleas of different species and permits calculation of the number of surviving parasites that are potential vectors of pathogens in endemic sites.

In our preceding article, we discussed the ability of rodents to brush out rat fleas--Ceratophyllus fasciatus (Nikitina, Nikolayeva, 1979). Our objective here was to determine differences in rodent behavior with regard to specific and nonspecific species of fleas. Such experiments had been conducted by Dubinin and Dubinina (1951) with rodents and fleas of Transbaykalia. They write that rodents rapidly eliminate flea species that are not specific for them; indeed, all of the experimental rodents (Mongolian bobak, suslik [ground squirrel], pika, narrow-skulled vole, guinea pig) rapidly anihilated the avian flea, Ceratophyllus gallinae. All rodents, with the exception of the bobak, destroyed the marmot flea, Oropsylla silantiewi, but such a distinct finding was not made in experiments with other flea species. In the experiments of Zhovtyy and Vasil'yev (1962), rodents destroyed more fleas of specific species in some cases and nonspecific in others. Thus, the bobak consumed more fleas of the specific species O. silantiewi (20% of all placed on it per day) than Ceratophyllus penicilliger (4%) and Ctenophthalmus assimilis (2.9%) species of small rodent fleas. The clawed gerbil consumed more of its own Frontopsylla luculenta fleas (20.3%) than suslik and bobak fleas: Ceratophyllus tesquorum--7.7%, O. silantiewi--3%. But there were also opposite instances. For example, the long-tailed suslik destroyed fewer suslik fleas (C. tesquorum--9.7%) than mouse fleas (Leptopsylla segnis--14.3%). Rats and gerbils consumed the rat flea (Xenopsylla cheopis) to the same extent (12%). The article of Zhovtyy and Vasil'yev offers no explanation for such differences.

We experimented with four rodent species: house mouse (Mus musculus), common (Microtus arvalis) and common redbacked (Clethrionomys glareolus) voles, and steppe lemming (Lagurus lagurus), as well as five flea species: Leptopsylla segnis, Cerathophyllus fasciatus, C. penicilliger, Ctenophthalmus uncinatus and Ct. assimilis; L. segnis, the specific flea of house mice, is confined

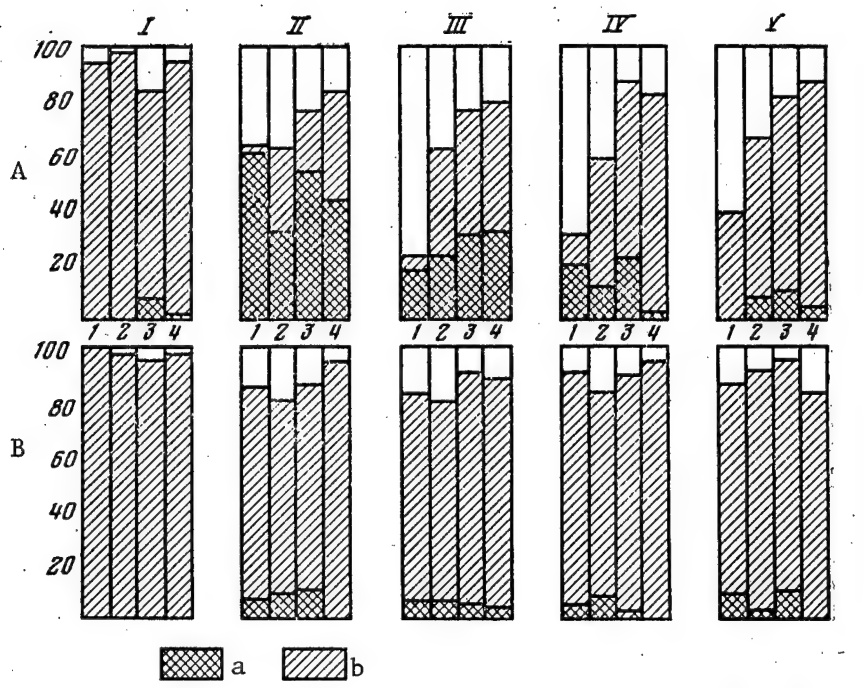
strictly to the host body, being localized chiefly on the head and along the spine. C. fasciatus is a rat flea but extremely eurybiontic: indoors it passes on to house mice rather frequently, whereas in its natural habitat is encountered in small quantity in gray vole burrows and is localized between the host's hind leg and near the tail. This species is less confined to fur than the preceding one, it is very mobile and rapidly jumps off the animal into the litter if the animal displays the slightest restlessness. C. penicilliger and Ct. uncinatus are redbacked vole fleas, and they are also encountered on other rodents inhabiting forests. Ct. assimilis is a common vole flea, encountered in large number in their nests, but in the presence of the host it prefers to stay on rodent fur. Vole fleas spread over the entire body of their host and are not very mobile.

To conduct experiments with one flea species, we took 20 specimens of each rodent species and placed a cardboard collar, which prevented them from scratching, on 10 animals (5 males and 5 females), but not on the other 10 (5 males and 5 females). The animals were picked in pairs of the same weight. We placed 10 fleas on each rodent. Thus, we used 800 insects in an experiment with fleas of one species. Since experiments were conducted with five flea species, we used a total of 4000 fleas. The total number of animals constituted 400. The rodents were kept in individual glass jars with wood shavings at the bottom. The animals and litter were examined 1 day after placing fleas on them.

If we consider the data on number of fleas remaining on specific and nonspecific hosts, we see that the findings are consistent for house mice. L. segnis is specific to house mice and is retained in larger number on them than are fleas of other species (94% of fleas placed on animals without collars). C. fasciatus, a rat flea, is definitely more inherent in mice than voles and is in second place (63%). Considerably fewer vole fleas are retained on mice: 38% Ct. assimilis, 30% Ct. uncinatus and 23% C. penicilliger.

However, we failed to demonstrate a tendency toward reduction in number of remaining fleas on other rodent species in the class of specific and nonspecific types of parasites. Specific species, C. penicilliger and Ct. uncinatus--76% and 86%--remained on redbacked voles without collars, but there were about just as many nonspecific species (76-83%). There were more fleas of the specific species, Ct. assimilis, on common voles without collars--87%--with about the same quantity of forest vole fleas (82 and 88%) and rat fleas (83%), and most of all there were house mouse fleas (94%). In the steppe lemming, all of the fleas were nonspecific, and there was the most retention on these animals without collars of house mouse fleas (98%) and other, also nonspecific species--58-66%.

The findings are more understandable if considered for different flea species (Figure). The house mouse flea, L. segnis, is little attacked by all four rodent species, and 83-98% remained on animals without collars versus 95-99% on those with collars. The rodents brushed very few fleas off on the litter. There were no appreciable differences between rodents of different species with regard to getting rid of these parasites, but the redbacked voles removed somewhat more of them (Figure).



Quantity of fleas retained on rodents and in litter 1 day after placement; each column represents 100 fleas on 10 rodents (10 fleas per animal)

A) rodents without collars
B) rodents with collars
I) Leptopsylla segnis
II) Ceratophyllus fasciatus
III) C. penicilliger
IV) Ctenophthalmus uncinatus
V) Ct. assimilis

1) Mus musculus
2) Lagurus lagurus
3) Clethrionomys glareolus
4) Microtus arvalis

a) fleas in litter
b) fleas on rodent

The rat flea, C. fasciatus, is not specific to any of the rodents used in the experiments, but, as we have stated above, it is quite euribiontic. Experimental rodents destroyed more of these fleas than house mouse fleas; after 1 day, 62-88% of the fleas survived on rodents without collars and 89-96% on those with collars. In the first group of rodents, most of the fleas (31-60%) had been brushed out on the litter and 3-31% remained in their fur. Rodents wearing collars brushed off fewer fleas: no more than 10% found in the litter, versus 80-94% on the animals. With respect to this flea species, there were evident differences in capacity of different rodent species to remove the ectoparasites, and they were ranked in the following order: mice, steppe lemmings, redbacked voles and common voles. Of the fleas placed on mice and lemmings, the same number survived, but the mice had brushed virtually all of them on the litter, only 3% remaining on the animals versus 60% found in the litter, whereas in lemmings, 31% of the fleas remained in their fur and 31% were found in the litter. The largest number of fleas was retained on gray voles (Figure).

The rodents displayed similar behavior with regard to vole fleas. Of the total number of fleas placed on animals without collars, after 24 h there

remained 24-78% of redbacked vole fleas, C. penicilliger, 30-86% Ct uncinatus and 38-87% gray vole fleas, Ct. assimilis. Mice destroyed the most fleas, followed by steppe lemmings, for whom these species are nonspecific, and even fewer were destroyed by redbacked and gray voles. In general, the same order prevailed, with respect to capacity to shed fleas, as for C. fasciatus, but somewhat fewer vole fleas were brushed off on the litter (Figure). Apparently, for these four flea species, the rodents' capacity to shed them is more significant than their specificity for these rodents. The confinement to a given host species in their natural habitat is determined more by the microclimate of the rodent habitats, to which arthropoda are very sensitive, than by the behavior of different animals.

When collars are placed on the rodents, the species-related differences in capacity to get rid of fleas are leveled off. There was a small difference between different rodents in number of surviving fleas (a maximum of 14%). As a rule, more fleas are retained on mice, which is indicative of their greater attraction as hosts. Similar conclusions were derived from studies with ticks (Nikitina, Zhmayeva, 1963; Lebedeva, 1978).

We checked the differences between destruction of male and female fleas. In our experiments somewhat more males perished than females, about 3-6% for different species. Statistical processing showed the differences to be unreliable.

We also tried to demonstrate behavioral distinctions of male and female rodents. There were no clearcut differences between the sexes with respect to number of fleas destroyed. Male steppe lemmings rid themselves of fleas somewhat better than females, the differences in number of remaining fleas of different species constituting 4-10%, but they were statistically unreliable. Male redbacked voles rid themselves better of vole and mouse fleas than females (the differences were also low--4-12%), however, females destroyed more rat fleas, C. fasciatus, than the males (by 4%). Male and female common voles behaved the same with regard to rat fleas; males destroyed somewhat more (by 4%) L. segnis and Ct. uncinatus than the females and, conversely, females destroyed more (by 2-6%) Ct. assimilis and C. penicilliger. These differences are no doubt coincidental, and one can consider that animals of both sexes behave the same. The more active specimens destroy more parasites, and there are somewhat more such males than female rodents.

Thus, in our experiments, there was no difference in rodent behavior with regard to specific and nonspecific flea species. The differences in number of fleas destroyed by rodents of different species were determined by their ability to brush themselves rather than specificity of the parasite. However, survival of fleas of different species is also related to the behavioral distinctions of the arthropods themselves. L. segnis, which are localized in groups on the host's head and along the spine, are virtually entirely retained on the rodent's body after 24 h. Many C. fasciatus survive due to their great mobility and jumping down in the litter when a rodent is restless. Vole fleas are less mobile, they spread over the entire rodent body and large quantities are destroyed.

We failed to demonstrate clearcut differences in male and female rodent behavior in relation to fleas. There were minor differences in number of dead male and female fleas, but still there is a tendency toward greater survival of females, as compared to males.

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SOCIO-HYGIENIC FACTORS OF BIRTH RATE AND FORMATION OF ABLE-BODIED POPULATION OF RSFSR

Moscow ZDRAVOOKHRANENIYE ROSSIYSKOY FEDERATSII in Russian No 1, Jan 82 (manuscript received 13 Mar 81) pp 25-28

[Article by N.A. Shneyderman, Republic Scientific Research Laboratory for Medical Demography (director--Doctor of Medical Science M.S. Bednyy) at the Moscow Scientific Research Institute for Epidemiology and Microbiology, RSFSR Ministry of Public Health]

[Text] Outlined in resolutions made at the 26th CPSU Congress as the most important goals for economic and social development of the country for the forthcoming five-year plan were the need to implement effective demographic policies, to strengthen the family as the most important unit of a socialist society, to create the best conditions for combining motherhood with active participation of women in work and public activities and to institute a system of measures to increase the life span of people by improving their health.[1] Fulfillment of these goals will be impossible without consideration of the most essential socio-hygenic factors which influence birth rate, the work capacity of the population and life span.

One of the most important demographic factors influencing birth rate is marriage rate, inasmuch as the marital birth rate is the basic component of the general level of propagation. At the present time, extramarital births represent a serious problem because the relative significance of them in terms of the total population of childbearing women has increased. Based on data from selected studies conducted in a number of territories of the RSFSR, the proportion of extramarital births varies to a significant extent. Thus, in 1978 in the cities of Voronezhskaya Oblast, this index was 3.6 percent, in the villages--5.9 percent; for the same year in Irkutskaya Oblast it was 18.4 and 24.6 percent, respectively; in Tuvinskaya ASSR--21.5 and 18.9 percent, respectively. We should note that in 1970, the number of extramarital births was less. The increase in the relative significance of extramarital births for the total population of childbearing women indicates that in many regions, as a result of an age-sex disproportion, selection of marriage partners is made difficult.

Analysis showed that extramarital births are most prevalent in a very young age group (15-19 year olds) and in women over 30. The least significant number of extramarital births was noted in the age group of 20-24 and the most in the age group of 40-44. In the cities of the RSFSR, as compared to the villages, the extramarital birth rate was lower in young age groups and higher in the group over 35 years. This phenomenon

is directly related to the characteristics of the age-sex structure of the population of the republic. According to data from the general population census, the sex disproportion in age groups up to 35 years was significantly less in the urban areas of the republic than in the villages; in the cities an increase in the disproportion was noted in the age group over 35 whereas in the villages this disproportion decreased.[2] Such an age-sex structure of the population is complicated by the effects of migration, the consequences of a higher death rate for men in comparison with that found for women, expressing a demographic echo from the War. Thus, extramarital births are a serious demographic and social problem towards which should be focused the attention of public health agencies so that demographic policies may be formulated. Despite a wide series of advantages made available to single mothers in our country, children born out of wedlock often grow up in worse conditions than those born to a married couple. A women giving birth to a child out of wedlock experiences stress which, obviously, affects the health of the child. The death rate of children in the age group of up to 1 year is lower for those born in wedlock than for those born to unwed mothers. Age-related factors have important significance. The concentration of extramarital births in the younger and older age groups of childbearing contingents is adversely expressed in the health of the mother and child.

A serious socio-hygienic problem which must be considered in developing demographic policies appropriate to different regions of the country is multiparity and problems related to short intervals between pregnancies; factors characteristic of reproductive practices in the majority of autonomous republics in the RSFSR. A committee of experts from WHO on protecting the health of mother and child concluded that multiparity, short intervals between births and pregnancy in the earliest or latest segments of the reproductive period increase the risk of birth complications.[3]

The majority of autonomous republics in the RSFSR have a relatively high birth rate. The relative significance of children born to mothers in the age group over 30 years is also high. At the same time, in the majority of urban settlements of the central regions of the RSFSR where the birth rate is especially low, the relative significance of children born to mothers older than 30 years of age is 2-3 times lower. In the villages, it is significantly higher than in the cities. In recent years in the RSFSR, a growth in the relative significance of children born to women in the age group of 15-19 years has been noted. In this regard, public health agencies must turn serious attention to interpreting the preferability (both for the health of the mother and for that of the child) of bearing children when a woman is in the 20-30 year age group.

An important aspect of increasing the birth rate is overcoming and developing therapy for infertility. According to the estimation of specialists, approximately 10 percent of all marriages in this country are sterile. Of these, 8 percent represent primary infertility and 2 percent are the result of secondary causes.[4] Secondary infertility is often the result of previous abortions. From this point of view, abortions during the first pregnancy and frequently repeated ones are most dangerous. Presently, infertility is a problem which is poorly amenable to therapy. However, by eliminating the most serious reasons for its cause, such as abortions, we can significantly decrease its frequency.

At the present time, artificial abortion is the most widespread method of contraception. Suffice it to say that in a number of territories of the RSFSR, the

correlation of births and abortions in 1979 exceeded 1:3, the lowest number of abortions was recorded in the autonomous republics of the Severo-Kavkazskaya Economic Rayon and in Tuva--regions where the birth rate is practically unlimited. The worse correlation between births and abortions was noted in Magadanskaya (1:3.3), Kamchatskaya (1:3.2) and Kaliningradskaya (1:3.0) Oblasts and other areas of the Zapadno-Sibirskiy Economic Rayon.

The danger of artificial abortions is determined not only by the degree of secondary infertility, but also by miscarriages, extrauterine pregnancies and complications during delivery and the postpartum period frequently experienced by women who have had abortions in the past. Thus, in the future, abortions will not only be a factor which lowers the birth rate directly but will also affect it indirectly by increasing the number of fetal detachments during pregnancy, the incidence of secondary infertility and gynecologic disorders which cause women to abstain from childbearing or put it off for an indefinite length of time.

It is now universally accepted that prohibition of artificial abortion by itself cannot lead to an increase in birth rate because abortion represents only the method used by families wishing to limit the number of children they have. An experiment conducted by the member countries of CEMA [Council for Economic Mutual Aid] showed that indices for birth rate usually increased only in the first years following prohibition of artificial abortion. Subsequently, without effective means of contraception or poor propaganda for them, women are forced to seek out criminal abortions, the dangers of which are constantly being reiterated. Nevertheless, a decreased incidence of abortion signifies improvement in the state of health of women of the reproductive contingent and their offsprings and a greater number of uncomplicated births. Therefore, the most important step for public health agencies, in relation to birth rate, must be the development, introduction and distribution of effective contraceptive methods, improvement in the health practices of the population including contraception and intensification of propaganda concerning the dangers of artificial abortion.

Birth rate is the most important factor influencing reproduction of a population when the death rate has stabilized. The dynamics of birth rate, death rate and migration over a significant period of time determines the age-sex structure of a population. In turn, the established age-sex structure of a population affects indices for birth rate, death rate, morbidity and invalidization of the population and, thus, the growth rate of the able-bodied population.

Territorial differences in rates of demographic processes in the RSFSR has led to the formation of varied age-sex structures in the population according to the regions of the republic. The extent of variation in relative significance of different age groups in the total population according to region, as well as cities and villages of one regions, is quite large. Thus, in the villages in the majority of oblasts of the Tsentral'niy, Tsentral'no-Chernozemniy and Povolzhskiy Regions, in which birth rate is not high, the proportion of able-bodied individuals in the total population is lower than average for the Russian Federation, expressing clearly the process of aging of the population. In the autonomous republics of the RSFSR, which are characterized by a high birth rate, a high relative significance of retired age groups with a low relative significance of able-bodied population has been noted. Given other comparable conditions, these regions offer better prospects for increase

in the contingents of able-bodied individuals in the future than regions with a low birth rate. However, we should keep in mind that multiparity leads to an increase in the perinatal and infant death rate, a decline in the health of women and removes them during the most valuable age, from an economic standpoint, from the ranks of the working reserves for an extended period of time. Thus, the process of reproducing the able-bodied contingents will be the most "economical" given a moderate birth rate. In the majority of oblasts in Siberia and in all oblasts of the Far East, the relative significance of populations in the able-bodied age group is higher than the average for the RSFSR; an effect of the direction and selectivity of migration. The village population of the Russian Federation, in demographic terms, is older than the urban one with a higher proportion of retired people and a smaller proportion of able-bodied ones. This phenomenon is also the result of the influence of 2 factors--migration of population and the dynamics of birth rate during the preceding 10-20 years. The aging of a population leads to a decrease in the rate of increase of the working contingents, increases the economic burden on the able-bodied population because of these dependents and represents a serious public health problem. Thus, the present age structure of the population in many territories of the RSFSR, characterized by a high relative significance of middle-aged and elderly age groups in the population, can be considered a regressive one and will not ensure a normal reproduction of the population.

These representative prospective calculations show that, given an unaltered regimen of reproduction, which constructed the age structure of the population of the RSFSR from 1970 to 1990, the proportion of population in the retired age groups in the cities of the republic will increase, there will be a slight increase of this age group in the villages, there will be further intensification of the process of aging of the population both in urban and rural localities and there will be a reduction in the proportion of population in the age group of 15-59 years. The rates of increase of the general numbers of population as well as of its able-bodied contingents will decline steadily. Such a prognosis cannot be viewed as an exact prediction--it is an estimation pointing to what this demographic situation will lead, given conditions for its long-term continuation.

An important questions for ensuring reproduction of able-bodied contingents is their age structure. In this regard, indices for the same population size have unequal value from an economic standpoint. B.Ts. Urlanis views age groups of an able-bodied population in relation to productivity of labor and the level of qualifications as seen in ranking the decline in the following pattern: 30-39 year olds, 20-29 year olds, 40-49 year olds, 50-59 year olds and 16-19 year olds.[5]

The relative significance of different age groups is also valuable for public health: the extent of specialized medical aid, losses related to morbidity, death rate, invalidization in various age groups of the population all differ. Analyses indicated that, given the continuation until 1990 of the current tendencies in birth rate and death rate, in comparison to patterns characteristic of 1970, a decrease in the relative significance of the most valuable, from an economic standpoint, age groups will occur (I and II groups according to B.Ts. Urlanis' classification) and the relative significance of elderly contingents in the total population of the RSFSR will also decline with some increase in these groups in the cities and a decrease of them in the villages.

We should consider that the population working in the national economy of a country depends not only on numbers of able-bodied individuals but also on the advantages made available by the state to working pensioners, the state of health of the population, the prolongation of the period of its working capacity and the death rate of the able-bodied contingents. Therefore, the work of public health agencies to decrease morbidity and death rate, to increase the period of working capacity of the population and to preserve the working reserves of the country must be intensified.

FOOTNOTES

1. "Osnovnye napravleniya ekonomicheskogo i sotsial'nogo razvitiya SSSR na 1981-1985 gody i na period do 1990" [Basic Directions for Economic and Social Development of the USSR in 1981-1985 and for the Period up to 1990], PRAVDA, 3 Mar 1981.

2. "Itogi Vsesoyuznoy perepisi naseleniya 1970" [Summary of All-Union Population Census for 1970], vol 2, Moscow, 1972, pp 18-19.

3. "New Tendencies and Approaches in the Field of Protecting the Health of Mother and Child," WHO, Geneva, 1978, p 29.

4. Stankov, A.G., "Bezdetnyy brak" [Childless Marriage], Tashkent, 1969.

5. Urlanis, B.Ts., "Voprosy ekonomiki" [Questions of Economics], 1970, No 5.

9139
CSO: 1840/133

UDC: 614.2(571.1/.6)

HEALTH PROTECTION PROBLEMS IN SIBERIA, THE FAR EAST AND THE FAR NORTH

Moscow ZDRAVOOKHRANENIYE ROSSIYSKOY FEDERATSII in Russian No 1, Jan 82 (manuscript received 10 Sep 81) pp 3-7

[Abstract] In the east and north, where special climate, geography, production and social conditions combine with high population mobility in remote areas, a number of new problems arise in population development control and public health. An important part of such programs must be population development including health preservation programs. These areas are highly urbanized with over 60% of the population in cities. The rural population is shrinking. Migration processes are dominant in the formation of the age and sex structure of these populations. The environmental impact of new technology and industries is also having a harmful effect on the health of these populations. This article calls for the broad scope of ministries and departments to take part in improving the health of the region by improving public health planning, devising specific measures for organization of medical assistance, development of a demographic policy which take in consideration public health and improvement of environmental quality. An important stage in the solution of many problems for these areas was the recently held conference on problems of health protection and organization of medical assistance in these regions. Many of the materials of this conference are published in this issue of the journal.
[132-6508]

UDC: 614.1:[312.6+574.2/.3]([47+57]-17)

MEDICAL-BIOLOGICAL ASPECTS OF STUDY OF HEALTH OF FAR NORTHERN POPULATION

Moscow ZDRAVOOKHRANENIYE ROSSIYSKOY FEDERATSII in Russian No 1, Jan 82 (manuscript received 10 Sep 81) pp 7-12

OREKHOV, K. V., Scientific Research Institute of Medical Problems of the North, Siberian Division, USSR Academy of Medical Sciences, Krasnoyarsk

[Abstract] The center of attention of modern medicine is the study of the health and disease of man as an integral biosocial object. The transforming

activity of man has now reached a planetary scale. The assimilation of new territory in the far north, Siberia and the far east has produced a highly mobile population, strengthened interpersonal and increased interpopulation contact and led to new antigen contact. The subject of medicine must be the study of the morphophysiological, psychoemotional conditions of optimal achievement of the inherent capacity of man. Health is no longer simply a biological category. Health of the population is an important indicator of social progress. The program "the north, the ecology of man in the far north," developed by the Institute of Medical Problems of the North, Siberian Division, USSR Academy of Medical Sciences, is therefore quite important. The health of the population is intimately related to the ecology of the region, its geographical and climatic peculiarities. The scientific and organizational prerequisites have now been created for the development of northern medicine. The unification and creation of effective scientific programs of joint research are required.
[132-6508]

UDC: 362.1([47+57]-17)

STATUS AND MEANS OF FURTHER IMPROVEMENT OF MEDICAL SERVICES FOR FAR NORTHERN POPULATIONS

Moscow ZDRAVOOKHRANENIYE ROSSIYSKOY FEDERATSII in Russian No 1, Jan 82 (manuscript received 10 Sep 81) pp 12-15

YUFEREV, S. V., Krasnoyarsk

[Abstract] Improvements in public health services for the half million persons in the far northern portion of Krasnoyarsk Kray during the 11th Five-Year Plan are described. The increases in the number of medical personnel, emergency air ambulance services, numbers of people who now receive periodical medical examinations and the availability of hospital bends are noted. Remaining shortcomings include the inability to provide emergency surgery quickly, and difficulties created by the huge territory and severe climate. The birth rate has increased and mortality decreased, particularly infant mortality. There are still problems. The standards for organization of public health services were developed for the middle regions of the RSFSR and are not always applicable in the far north, where severe climate and geographic conditions require larger numbers of medical personnel, hospital beds and other types of medical services. Maintaining public health requires further work in these areas.
[132-6508]

UDC: 362.1(571:12-17)

ORGANIZATION OF MEDICAL ASSISTANCE TO FAR NORTHERN TYUMEN' OBLAST

Moscow ZDRAVOOKHRANENIYE ROSSIYSKOY FEDERATSII in Russian No 1, Jan 82 (manuscript received 10 Sep 81) pp 15-18

KRETININ, V. N., Tyumen'

[Abstract] The rapid economic development of autonomous okrugs resulting from the discovery of oil and gas has been accompanied by great social and demographic changes. The population of the okrugs has increased by a factor of 2.9 during the 1970's. The structure of morbidity is specific for the area, with the most frequent cause for visits to the physician being respiratory disease, followed by infectious and parasitic diseases, nervous system diseases and skin disease. The structure of public health institutions in the area is briefly described. There is a particularly great contrast between health services available to urban and rural residents. More effective and simpler diagnostic tools are needed under the rural conditions in such northern areas to allow more effective health services without requiring large investments in equipment.
[132-6508]

UDC: 614.2(470.11)

STATUS AND PROSPECTS FOR DEVELOPMENT OF PUBLIC HEALTH IN NENETSKIY AUTONOMOUS OKRUG OF ARKHANGEL'SK OBLAST

Moscow ZDRAVOOKHRANENIYE ROSSIYSKOY FEDERATSII in Russian No 1, Jan 82 (manuscript received 10 Sep 81) pp 18-20

DUBCHENKO, M. V.

[Abstract] The Nenetsk autonomous okrug includes 147,000km^2 and a population of 49,000 persons. Winter lasts from 200 to 260 days here, and most people live in small villages of 40 to 1200 persons, tied together by aviation and, briefly in summer, by water. The okrug has nine hospitals and 460 beds, 115 of which are in rural areas, plus an okrug tuberculosis preventive dispensary of 105 bends, five rural dispensaries, 31 obstetric points, a 35 bed children's home, an okrug sanitary-epidemiologic station and 8 dispensaries. There are 21.2 physicians and 87.3 paramedical personnel per 10,000 inhabitants. Tuberculosis morbidity has been decreased by a factor of 3.6, helminthosis by a factor of 2 in the past 10 years. Mobile medical detachments have been serving the population for 20 years. A program of scientific research work has been started to study living conditions and public health in this far northern area.
[132-6508]

UDC: 362.1(571.651)

ORGANIZATION OF MEDICAL SERVICES FOR CHUKOTSK AUTONOMOUS OKRUG

Moscow ZDRAVOOKHRANENIYE ROSSIYSKOY FEDERATSII in Russian No 1, Jan 82 (manuscript received 10 Sep 81) pp 20-23

GVAGVALIYA, M. P.

[Abstract] In contrast to the tragic situation before the revolution, there are 257 medical institutions with 2335 beds, 40 traveling obstetric points, 38 milk kitchens including 23 in rural areas in Chukotka today. All persons living in the area, even in tiny villages, are provided with health services. The most important task of the future is development of ambulatory polyclinic services, reducing the size of territorial physician-supported uchastoks, increasing the number of uchastok physicians and improving the equipment with which they are supplied. Praticing physicians in the far north must continue to work on developing problems of preservation of the health of the population and organizing medical assistance. The creation of a permanent institutional subdivision for combined study of problems of public health in the population of the far north, considering the demographic and medical factors there present, will allow solution in the immediate future of the problems of prevention and decreasing morbidity and mortality of the population, development and introduction of modern methods of therapy of the pathologies most common in the district.
[132-6508]

UDC: 362.1(571.66)

STATUS OF MEDICAL ASSISTANCE TO POPULATION OF KORYAKSKIY AUTONOMOUS OKRUG OF KAMCHATKA OBLAST

Moscow ZDRAVOOKHRANENIYE ROSSIYSKOY FEDERATSII in Russian No 1, Jan 82 (manuscript received 10 Sep 81) pp 24-25

KUTNYAKOV, V. Ye., candidate of medical sciences

[Abstract] The Koryakskiy okrug has 32 hospitals and preventive dispensaries with a total of 1155 beds, or 33 beds per 1,000 inhabitants. There are 66.7 physicians and 190 paramedical workers per 10,000 population. Over the past twenty years, tremendous changes have occurred, but the conditions of health of the minority populations in northern Kamchatka have continued to be somewhat disturbing. Villages are being eliminated by resettlement, a process continuing today. This radical solution to the problem of small villages will allow the solution of many problems such as nutrition, preservation of traditional industries and conservation of public health.
[132-6508]

UDC: 614:312.313

SOCIAL-DEMOGRAPHIC CHARACTERISTICS OF DIVORCED PERSONS (BASED ON DAGESTAN)

Moscow ZDRAVOOKHRANENIYE ROSSIYSKOY FEDERATSII in Russian No 1, Jan 82 (manuscript received 10 Apr 81) pp 29-31

LASHTAYEVA, G. Ya., Department of Social Hygiene and Organization of Public Health, Dagestan Medical Institute, Makhachkala

[Abstract] A special combined social-hygienic study of the families of divorced persons in Makhachkala was undertaken to study the conditions of life, reveal the reasons for breakup of the families and develop medical-social classifications for divorce motivations. (Dagestan is a multi-national republic whose population profess the Moslem religion--due to religious tradition marriages are entered into, and divorces occur, without official recording and this does lower factual indices.) The materials of the study included data on all divorces recorded in Makhachkala between 1972 and 1973 (1408 divorces). In the second stage of the study, a special questionnaire was used to collect information on changes in social-hygienic conditions of life and health in these families during the five years after the divorce. The influence of relative age, particularly very young age of wives, length of time married and national origin was traced. Mixed nationality marriages ended in divorce 2 1/2 times less frequently than marriages within the same nationality.
[132-6508]

UDC: 616-074/-078:061.6:[378.661+61:061.62](571.1/.6)

ROLE OF COORDINATION COUNCIL OF MEDICAL SCHOOLS AND SIBERIAN RESEARCH INSTITUTES IN IMPROVEMENT OF ACTIVITY OF CENTRAL SCIENTIFIC RESEARCH LABORATORIES

Moscow ZDRAVOOKHRANENIYE ROSSIYSKOY FEDERATSII in Russian No 1, Jan 82 (manuscript received 7 Apr 81) pp 31-32

GOL'DBERG, Ye. D., professor, Tomsk Medical Institute

[Abstract] Particularly significant in the increased utilization of the scientific research potential of educational institutions is the network of Central Scientific Research Laboratories (CSRL) at these schools. Siberia and the Far East has 11 medical institutes [i.e., medical colleges]. Such CSRL are functioning in nine of these medical institutes and in the Novokuznetsk Institute for the Advanced Training of Physicians. Three of the institutes have no such laboratories. Over 400 workers, including 50 Candidates of Sciences, are studying in these CSRL. This tremendous scientific force must be intelligently used. The Coordination Council takes very seriously the task of increasing the qualification of Central

Laboratory fellows and provides extensive opportunities for exchange of experience and continuing education. The council believes that further improvement of the work of the Central Laboratories will require a stimulus to ongoing training of doctors of sciences and candidates of sciences among the scientific fellows in the laboratories.
[132-650]

UDC: 616.21/.22+616.28]-036.12:313.13(470.311-25)

DISTRIBUTION OF CHRONIC DISEASES OF UPPER RESPIRATORY TRACT AND OF HEARING ORGAN AMONG ADULTS AND ADOLESCENTS IN MOSCOW

Moscow VESTNIK OTORINOLARINGOLOGII in Russian No 2, Mar-Apr 82
(manuscript received 6 Oct 81) pp 55-58

KUZNETSOV, V. S., professor, VOZNESENSKAYA, I. A., doctor of medical sciences, MOROZOV, A. B., candidate of medical sciences, and PETROVA, M. V., Moscow Scientific Research Institute of the Ear, Nose and Throat, RSFSR Ministry of Health

[Abstract] An in depth study of the distribution of chronic diseases of the upper respiratory pathways and of hearing in adults and adolescents living in Moscow was undertaken. This was a part of a combined study to determine organizational forms and required volume of therapeutic and diagnostic work as factors in transition to dispensary services for the entire population. The study was performed jointly under the methodological leadership of the All-Union Scientific Research Institute of Social Hygiene and the Organization of Public Health. The preliminary data produced indicate that urbanization has a very complex and varied influence on morbidity of the population and the distribution of upper respiratory and ear disease. Sufficient data were collected for utilization by public health services in both Moscow and other large cities for planning and organization of ENT support of the population. References 4 (Russian).
[131-6508]

UDC 612.87.014.477-064].014.421

ELECTROMETRIC INVESTIGATION OF HUMAN GUSTATORY ANALYZER UNDER NORMAL CONDITIONS AND IN SIMULATED WEIGHTLESSNESS

Moscow VESTNIK OTORINOLARINGOLOGII in Russian No 2, Mar-Apr 82
(manuscript received 16 Jun 81) pp 15-17

[Article by I. Ya. Yakovleva, doctor of medical science, Institute of Biomedical Problems, USSR Ministry of Health, Moscow]

[Text] Experience with medical provision for space flights has shown that altered perception of the taste of food has taken place in a number of cosmonauts. Similar phenomena were also observed in healthy individuals when some of the effects of weightlessness were simulated on Earth. Investigation of the gustatory analyzer during antiorthostatic hypokinesis (AOH) using sweet, salty, sour and bitter solutions, revealed changes in the threshold of sensitivity, the functional mobility of the receptors of the tongue and the gastrolingual reflex (V. Yu. Kurlyandskiy et al., 1974). Yet, in connection with features of the behavior of fluids in weightlessness, and, at the same time, the methodical complexity and labor intensity of investigating taste using solutions, these three methods are difficult to use in space flight. Electrometrical investigation of taste, which has been successfully employed clinically and in physiology (Yu. T. Mironenko, 1969; E. I. Zlotnik and I. A. Sklyut, 1970; N. S. Blagoveshchenskaya and N. Z. Mukhamedzhanov, 1978; Krarup, 1958; Pulec and House, 1964; Nilson, 1977; and others), made possible rapid determination of the quantitative status of taste reception. Electrometrical investigation of taste was used to study the gastrolingual reflex and apparently can be performed by the subjects themselves. In this connection, the method is of interest for space medicine.

We are reporting the data of electrometric investigation of the gustatory analyzer in 62 healthy men aged 25 to 45 years. The threshold sensitivity of the gustatory receptors of the right and left halves of the tongue was determined before, and 10-15 minutes after, intake of food. The difference in thresholds before and after eating constituted the quantitative index of the gastrolingual reflex. In connection with differences in instruments used for electrogustometry and the variability of norms according to the data of a number of authors (N.S. Blagoveshchenskaya and N. Z. Mukhamedzhanov, 1978; Krarup, 1958; and others), it became necessary to define more precisely the limits of physiological distribution of the thresholds of taste. The available literature does not give a quantitative characterization of the gastrolingual reflex determined by electrometric method.

Norms were determined in 53 persons. Nine persons were investigated under conditions of strict bed rest with the head of the bed inclined to -8 degrees. Feeding, hygienic procedures and physiological functions were performed without disturbing the subjects' body position. The duration of antiorthostatic hypokinesis was 5 days. According to current concepts, AOH is one of the effective terrestrial models of weightlessness and to a certain degree makes it possible to reproduce redistribution of blood and fluid media of the organism to the upper half of the body, to alter the activity of the afferent systems and other reactions of the organism which are characteristic of the acute period of human adaptation to weightlessness. Investigations of the gustatory analyzer were conducted prior to AOH, on days 1, 3 and 5 of bed rest and on the first day after its completion.

In order to investigate taste, we used an electrogustometer-1 instrument manufactured by the PPR [Polish People's Republic] Military Institute of Aviation Medicine, developed on the basis of a clinical instrument manufactured in the PPR and on the medical and technical recommendations of specialists from the USSR Ministry of Health IMBP [Institute of Biomedical Problems]. The methods of the investigations, modified for performance by the subjects themselves, are as follows.

After the instrument was connected to the electrical network the subject opened his mouth and gently rested his tongue on the lower lip. The subject applied the bulb of the active electrode to the right half of the tip of the tongue, 1-1.5 cm from the median line; in his left hand he held the passive electrode. When the knob of the passive electrode was pressed a direct current of gradually increasing strength entered the tongue. The occurrence of a slight sensation of acidity or slight pain on the tongue at the place where the bulb of the active electrode was applied corresponded to the threshold of sensitivity of the gustatory receptors to electrostimulation. At the same time the subject released the button of the passive electrode, removed the active electrode from his tongue, and by pressing the keyboard of the front panel of the instrument received a depiction of the threshold values of current strength in microamperes on the digital display board.

An analogous study was conducted on the left half of the tongue. The interval between exposures was 1 minute. The results of measurements were recorded in a log. In order to obtain average data, 5 measurements were conducted on each half of the tongue.

Before the work began, the subject mastered the methods of electrogustometry and conducted 4-6 familiarizing and training measurements at an interval of 2-3 minutes until sufficiently accurate reproduction of the indices being studied were obtained. Training and all subsequent measurements of gustatory sensitivity were performed under a physician's control. The results were subjected to statistical analysis.

When the norms of gustatory sensitivity were determined in the group under investigation, pronounced variation of data was observed. The electrogustometry indices fluctuated from 13 to 65 μA, and as a rule were symmetrical (divergence of 2-4 μA). However, in much the same way as the

results recorded by Krarup (1958) the data of repeat measurements of the threshold of taste in one and the same individual taking into consideration diurnal periodicity, indicated sufficiently accurate reproduction of the results obtained. According to average data, the threshold sensitivity of taste receptors to electrostimulation on the right half of the tongue was 32.9 ± 2.64 μA, on the left, 29.5 ± 2.58 μA ($P<0.01$).

Food intake in all subjects resulted in a significant rise in the threshold of taste; this corresponded to normal interaction of the gustatory reception of the tongue and the stomach, established by other investigators (N. S. Zayko, 1956; D. S. Voskanyan, 1973 and others). An increase in the threshold of taste after eating was characterized as a positive gastrolingual reflex; a decrease, as negative.

In our investigations, the threshold of taste after eating (average data) for the right half of the tongue was 39.1 ± 3.30 μA; for the left, 32.8 ± 2.27 μA ($P<0.01$). According to average data the threshold of taste after eating increased by 6.2 μA on the right and by 2.3 μA on the left, or by 18.8 and 7.8 percent. The overall pattern of the reactions persisted--a relatively lower threshold of taste on the left half of the tongue.

In the group of individuals exposed to the influence of AOH, in the background study the threshold of taste corresponded to the norms obtained on the instrument used and were 34.6 ± 1.8 μA for the right half of the tongue and 33.5 ± 2.1 μA for the left. During the first day of bed rest, a statistically significant increase in the thresholds of taste, exceeding physiological deviations, was noted. The threshold of taste was 69.3 ± 0.8 on the right and 67.8 ± 1.6 μA on the left ($P<0.01$). In the overall pattern of the reaction, individual variability of the thresholds of taste was pronounced. Thus, when the background sensitivity of the gustatory receptors of the tongue are taken as 100 percent, the threshold of taste on the first day of AOH changed from 149 to 234 percent of the original level.

Throughout the entire period of AOH, the thresholds of taste remained high, but a tendency to their normalization was noted. On the fifth day the influence of the threshold of taste was 45.2 ± 0.9 on the right and 43.9 ± 1.5 on the left.

During bed rest, the influence of food intake on the threshold sensititivity of the gustatory receptors changed. As a rule eating was accompanied by a drop in the thresholds, and the pattern of the gastrolingual reflex changed accordingly. Thus, on the first day of AOH, a negative gastrolingual reflex was detected in 7 of 9 subjects, i.e., food intake caused a 1.5-5.4 μA or 5-16 percent drop in the thresholds. In two individuals food intake had no effect on the thresholds of taste--suppresion of the normal reflex set in. At the end of AOH the threshold of taste and the indices of gastrolingual reflex had reached the background values.

As a result of the investigations conducted, the limits of the physiological variation of the threshold sensitivity of gustatory receptors of the tongue to electrostimultion and the parameters of the gastrolingual reflex for men aged

25 to 45 years have been established. The possibility of the performance of electrometrical investigations of taste by the subjects themselves has been demonstrated. In much the same way as investigation of taste by solutions, electrogustometry in simulated weightlessness made it possible to observe changes in the activity of the taste analyzer, characterized by a significant increase in the thresholds and change in the gastrolingual reflex. Both the static plethora of the head and the change in the regulatory function of the central nervous system may be of significance in development of the detected disorders. Analysis of the data of the investigations has made it possible to recommend electrogustometry for investigation of the human gustatory analyzer under conditions of space flight. Electrometrical investigation of the gastrolingual reflex, which makes it possible to determine rapidly the quantitative influence of gastric digestion on gustatory reception, is of practical significance for a number of disciplines of clinical medicine.

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CADASTER OF ENDEMIC SITES OF TICK-BORNE ENCEPHALITIS

Moscow ZHURNAL MIKROBIOLOGII, EPIDEMIOLOGII I IMMUNOBIOLOGII in Russian No 11, Nov 81 (manuscript received 19 Jan 81) pp 93-99

[Article* by E. I. Korenberg, Institute of Epidemiology and Microbiology imeni Gamaleya, USSR Academy of Medical Sciences, Moscow]

[Text] We previously proposed to prepare unified national cadasters [registers] of endemic sites of human diseases [17]. By analogy to the water cadaster of the USSR, national land cadaster and certain other cadasters, the preparation of which is presently under discussion, we use the term of cadaster of endemic sites to refer to a summary of data tabulated in the same manner that would be mandatory for all planning and State agencies, pertaining to endemic sites of a specific infection (invasion), necessary for evaluation of their epidemiological potential and real epidemic hazard, as well as for planning preventive and therapeutic measures. The subject of such a cadaster and, consequently, objective of necessary assessment and documentation, should be an individual endemic site as a very concrete complex parasitic system, which occupies a specific part of the land and is spatially circumscribed [12, 15, 22]. In its final form the cadaster is an atlas of maps, which must show the boundaries of different sites and their numbers, as well as have a textual part that contains unified and similarly presented information about each site under the corresponding numbers. In essence, this should be the identification paper for an endemic site, containing necessary and sufficient information to implement the main end purpose of the cadaster. The requirements referable to the contents of such information were formulated in general terms previously [17]. In this article, an effort is made to define them as they relate to cadastral and assessment data on tick-borne encephalitis.

It was shown relatively recently that the boundaries of an endemic site of tick-borne encephalitis coincide with the boundaries for the population of the main vector of the virus. Endemic sites can be demonstrated over a large area by the cartographic method, according to spatial and isolating barriers

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by means of topographic maps [12, 14]. According to different researchers [6, 12, 15, 22, 30], an endemic site of tick-borne encephalitis (TBE) occupies an area of a a few to hundreds of square kilometers, depending on the landform conditions. Current conceptions of an individual endemic TBE site and previously expounded general theses on the objectives of cadastral studies enable us to discuss in greater detail the question of content of assessment data necessary to form a cadaster of endemic TBE sites, although in general many aspects of this problem still require comprehensive work.

The number given to an endemic site should help in rapidly finding it on the map and comparing cartographic and evaluation data. At the same time, it is desirable to adopt a numbering system for endemic sites that does not require that a specific order be followed in conducting cadastral work and permits conducting it simultaneously in different parts of the area of distribution of TBE virus. According to our conceptions, the range of the virus consists of 69 endemic regions. A specific name and number were assigned to each of them [13, 14]. Evidently, an endemic region consists of hundreds of endemic sites, and within the range of the pathogen they probably total several tens of thousands [12, 14]. On this basis, we propose for cadastral purposes that the number of any endemic TBE site consist of five digits: the first two could refer to its endemic region and the last three to the sequential number assigned to the endemic site when relevant studies were pursued in this endemic region. For example, the aggregate of endemic TBE sites in the interfluvial area between the Vyatka and Kama Rivers constitutes the Vyatka-Kama endemic region, to which No 20 was assigned in the system of zoning the geographic range of the virus [13, 14]. A map was charted for endemic sites in part of this endemic region, and a specific sequential number was assigned to each of them [12, 14]. To illustrate the theses advanced in this article, we chose endemic sites numbered 147 and 191. They should be given the numbers 20.147 and 20.191, respectively, in the cadaster of endemic TBE sites. The endemic sites referred to by this system as 61.014, 61.063 and 61.108* (see Table) are in the Badzhal'skiy endemic region, to which the number 61 was assigned in the system of zoning the range of the virus [13, 14].

The location, boundaries, size and landform features of an endemic site must be reflected in its cadastral and assessment document, and the description of these features, which appear to be simple at first glance, should be standardized as much as possible. The topographic map, on which the endemic site will be designated by its appropriate number, contains abundant information. For this reason, it is apparently sufficient to indicate in the explanatory text the part of an administrative rayon (or rayons) in which the site is located, as well as the name of a larger administrative entity to which this rayon belongs (see Table).

An endemic TBE site may be surrounded by territory where it is impossible for the pathogen to exist, or by a body of water (island site). In many cases, an endemic site occupies only part of a vast forest and borders on one (or

*We have arbitrarily given these endemic sites the numbers 14, 63 and 108, as an illustration of the proposed numbering procedure.

more) other endemic site along natural frontiers. This is an extremely important feature, in both the biocenotic and practical respects, and it must be reflected in the document for the site. In addition, there must be indications of its boundaries, the description of which is based on the geographic names appearing on the topographic map, and total area of the site (in square kilometers).

The landform, forest typological, geobotanical and certain other special studies usually describe the territory in great detail [7, 32]. In our opinion, in cadastral records, such a feature should be described as tersely as possible, reflecting only the most important elements of the site. We include among them the general nature of relief (smooth, hilly, mountainous, gullied, etc.; for mountainous regions--steepness and exposure of main slopes), absolute altitude of the locality, elevation gradient and general nature of vegetation. All this information is contained in a sufficient amount for cadastral purposes on the topographic map and, consequently, can be obtained without any special field studies.

The lemological potential is the most important feature of an endemic site. Whether or not people are present, it characterizes the potential danger of their being infected due to the profusion of direct sources of infection and conditions for transmission of the pathogen [26]. This term, which does not require replacement in our opinion, is absolutely identical in meaning to the verbose expressions proposed later on for TBE sites, "potential epidemiological significance" [28] and "potential epidemiological valency" [34].

Like several other researchers [1, 28, 29, 34], we [17] include among the main elements of the lemological potential of an endemic TBE site the number of I. persulcatus and I. ricinus imagos and extent to which they carry the virus. The danger of onset of not only transmissive, but ultimately alimentary infections depends on these indicators [8, 21].

Beklemishev [2] stressed that an endemic TBE site depends most on the population of the main vector, which could be designated as the principal element of a complex three-element parasitic system. As we know, I. persulcatus or I. ricinus ticks can be such a prime element in various endemic TBE sites. Both these species are significant over a certain, relatively small part of the pathogen's geographic range. It is desirable to mention the differences in species composition of vectors, manifested usually on the level of groups of sites, classes of sites or even endemic regions [15], when describing these entities of zoning the range of the pathogen.

The number of main vectors is the most significant factor that affects the epizootic process and epidemic manifestation of endemic sites. At the same time, it is one of the parameters that one can determine. It must be borne in mind at the outset that for cadastral and assessment purposes, it is not the perfectly accurate mean number of ticks, but its rank (level) that is ultimately of importance.

We know that the tick population may vary in size in different years. The natural fluctuation that is observed usually conforms to certain rather narrow and relatively stable "limits," which are inherent in a given population [18].

Examples of cadastral-assessment description of some endemic sites

Number of endemic site	Location	Degree of isolation	Boundaries	Area km^2	Principal landform features	Average quantity of main vector*	Tick viruliferousness, %	Average visits per 10 km^2	Average cases of infection in site (per 10 km^2)**
20.147	Southwestern Zav'yalovskiy and northern Malopurginskiy Rayons of Udmurtskaya ASSR	Contiguous	Edges of forest, Izh and Yaganka Rivers	120	Hilly relief; 80-180 m altitudes; linden-fir-spruce, severely deteriorated forests	7.5	0.5	≈550	0.6
20.191	Southern Alnashskiy Rayon of Udmurtskaya ASSR	Insular	Edge of forest	32	Gullied; 120-200 m; spruce-linden forests	13.4	?	≈1700	2.3
61.014	Central Verkhnebureinskiy Rayon of Khabarovskiy Kray	Contiguous	Urgal, Bureya, Malyy Iorik Rivers	972	Intermontane trough; 250-600 m; larch with mari*** and dark coniferous-poplar forests in valleys	2.3	0.2	<10	<0.002
61.063	Southeastern Verkhnebureinskiy Rayon of Khabarovskiy Kray	"	Suluk River & Suluk-Amgun' interfluve	459	Mountainous; 350-1400 m; western exposure of main slopes; larch, in valley dark conifer [boreal]-poplar forests	1.6	1.1	<1	-
61.108	Eastern Verkhnebureinskiy Rayon of Khabarovskiy Kray	"	Amgun', Temga Rivers & Suluk-Amgun' interfl.	648	Mountainous; 350-1400 m; eastern exposure of main slopes; rhododendron-reed larch forests	10.5	2.0	<1	-

*In these cases, F. persulcatus per flag-h. Specific data on quantity and viruliferousness of ticks were prepared from several sources [11, 16, 20, 31].

**Data on contact of public with endemic sites and number of cases of infection were calculated on the basis of previously published [16, 23, 24] and our own data.

***Stretches with numerous knolls or ridges and swampy patches between, or shallow bog.

For this reason, one can obtain data for any year about the overall number of ticks characterizing a given endemic site. But one must adhere to a mandatory condition, which is the uniform distribution of the representative number of records (record units) for the territory in question. At present, determination has been made of the correlation between the main record units for plain [25] and mountain [10] regions: number of ticks per flag-h and flag-km. This makes it possible to list any of the above parameters in the cadastral description of an endemic site.

The most important and comparable indicator of extent of carrying the virus in its main vector--individual infection of adult I. persulcatus and/or I. ricinus--was calculated by the method of Beklemishev [5]. The interval between minimum and maximum individual infection of ticks known in the geographic range of the virus is generally relatively small, but quite perceptible [27]. It is a known fact that degree of viral carriership fluctuates in different years in any endemic site. For example, as shown by many years of special observations in sites in the south of Kirovskaya Oblast and Udmurtskaya ASSR [31], <0.1 to 1.2% of adult tayga ticks carry the virus in different years. For this reason, in the cadastral description of vector infection, it is best, in our opinion, to use representative indicators that are the averages over a period of many years.

One should not confuse the indicators of epidemic manifestation of an endemic site with indicators that are used for epidemiological descriptions of pseudo-endemic TBE sites. Unfortunately, in the vast literature on TBE epidemiology, including special publications dealing with methods of epidemiological analysis [1, 29, 33, 34], a clearcut distinction is not made between these approaches, which differ in their objectives. At best, reference is made to the epidemiological features of pseudo-sites that are "coordinated" [conjugated?] with an endemic site [35]. Yet, the percentage of inhabitants who have been in a forest, the share of individuals bitten by ticks, morbidity in intensive indicators, relative number of individuals with some antibodies or other in blood and a number of other parameters recommended by authors [29, 33] and used most often in such cases describe pseudo-sites. But they only permit indirect evaluation, and to a small extent at that, of the scale of real epidemic manifestation of a given endemic site as compared to other sites. For this reason, as properly stressed by Beklemishev [3, 4], studies of pseudo-sites should proceed concurrently with the characteristics of endemic sites because of which the inhabitants of pseudo-sites become infected.

With regard to the problems discussed here, probably the most relevant is the idea advanced in a most general form of the need to identify the places where people are infected [33, 35]. However, assessment and comparison of epidemic manifestation and hazard of endemic sites should be made on the basis of distinct quantitative indicators, which provide an idea not only about the actual incidence, but potential incidence of infection on the territories of such sites. We consider the following two to be among such most important indicators: average (mean for many years) number of visits by people to an endemic site during the period of tick activity and average (mean for many years) number of sick people infected with TBE on the territory of this site. Since endemic sites differ appreciably in area, both indicators should be reduced to its particular unit. On the basis of conceptions of

possible sizes of different endemic TBE sites (see above), it is apparently expedient to calculate them per 10 square km. These parameters have been used separately in some form or other previously by some researchers, mainly in planning preventive measures for areas with a high and relatively stable population density [9, 19, 24, 34]. When considered together and listed in the cadastral and evaluation description, they enable us to understand why a given incidence of infection is observed with a given lemological [or lemic] potential, and how it can change with change in intensity of contact between the public and the endemic site. The latter is particularly important in uninhabited territories that are to be submitted to economic development. If the area of the site is known, when necessary this information can be used to calculate certain other parameters, for example, the absolute base figures for frequency of visits and incidence of disease, correlation between number of visits and number of sick cases, regardless of area of the site, etc.

As we know, alimentary cases of TBE are due to grazing and infection of goats in an endemic site. In our opinion, such cases of illness can be calculated just like transmissive ones to describe the epidemic manifestation of the endemic site. But contact of the public with the pathogen of TBE in the presence of goats occurs not only in the endemic site, but directly in pseudosites. The intensity of the latter could be estimated approximately by an additional parameter, the mean number of goats per 1000 population of a given locality. In particular, during the years of our studies, there were about 65 goats in endemic site 20.147 and 80/1000 population in site 20.191. In the last decade, there has been a drastic decline in overall incidence of alimentary infections in our country in view of the drastic reduction in number of goats. At present, only isolated cases of TBE are recorded in different regions, and they are related to consumption of goat milk. In view of the trends in development of modern livestock breeding, it can be assumed that the problem of alimentary TBE infection will not occur in the future on its former scale. For this reason, we did not include indicators of number of goats in the table of unified cadastral and assessment description.

Preparation of a national cadaster of endemic TBE sites would make it possible to undertake a general plan for decontaminating them, the need for which was indicated by Beklemishev [3]. Actually, we have yet to commence work on gathering cadastral and assessment information. Deployment thereof on a wide scale will make it necessary to prepare comprehensive instructions for methods to be used. For this reason, further discussion of the means of unifying [standardizing] cadastral and assessment studies is believed to be a particularly important task at expressly this time.

Conclusions

1. Preparation of a single national cadaster of endemic sites of tick-borne encephalitis is a mandatory prerequisite and most important phase of preparation of a general plan for the prevention of this infection.

2. The cadastral and assessment characteristics of each endemic site of tick-borne encephalitis should include (as the minimum) its number, a

description of its location, boundaries and size, brief landform features, main indicators of lemological potential and epidemic manifestation.

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TRANSPLANTATION OF SECOND HEART INTO CHEST IN MODELING ACUTE CARDIAC INSUFFICIENCY OF RECIPIENT

Moscow ANESTEZIOLOGIYA I REANIMATOLOGIYA in Russian No 6, Nov-Dec 81 (manuscript received 18 Mar 81) pp 45-48

MINCHENKO, B. I., Institute of Cardiovascular Surgery imeni A. N. Bakulev, USSR Academy of Medical Sciences, Moscow

[Abstract] The goal of the present study was to develop an experimental model for transplantation of a second heart suitable for chronic experiments. The hemodynamics and contractile function of both hearts during the first 48 hrs after surgery was studied. Thirty five chronic experiments were done on dogs. The recipients weighed 23.5 kg, the donors--13.7 kg. In eight experiments surgical technique was developed. After a successful surgery and adequate protection of the myocardium, the transplant functioned quite effectively. The period between the isolation of the donor heart and its inclusion in blood circulation of the recipient was crucial. After a short period of parallel performance, acute cardiac insufficiency was created by constriction of the ascending aorta of the recipient. Blood from the left ventricle of the recipient entered the donor's heart and led to an overall improvement of function. As a rule, both hearts performed asynchronously by a contrapulsation regimen. The transplanted heart was able to support effectively the "affected" heart for the first 48 hrs. The weakest link in the proposed model is the interatrial shunt and increased mobility of the transplanted heart in the chest cavity. Figures 4; references 12: 4 Russian, 8 Western.
[127-7813]

UDC 616.12-089.28:681.326

CONTROL SYSTEM 'SINUS' FOR ARTIFICIAL HEART

Moscow MEDITSINSKAYA TEKHNIKA in Russian No 6, Nov-Dec 81
(manuscript received 13 Mar 81) pp 23-25

GUS'KOV, I. A., ZATYURYUKIN, A. B. and KHMELEVSKOY, L. Ye.

[Abstract] A control system, titled "Sinus", was developed for operation of an artificial heart. The goal was to make it possible to obtain pressure pneumopulses of any desired form. This was achieved through servomechanisms which made it possible to change air pressures at the system outlet in relation to the controlling electrical signal. The system is capable of forming pulses with following parameters: pressure may be varied between 0 and 250 mm Hg for the left channel and from 0 to 150 mm Hg for the right channel during the systole; during diastole, it can be varied from -50 to 10 mm Hg for both channels. The duration of systole may be regulated accordingoto the function $\tau_s = KT$ or $\tau_s = K\sqrt{T}$ (K varies from 0.2 to 0.6). The frequency of pulses may reach up to 150 beats per minute. In animal experiments an artificial heart "Modul'" coupled to "Sinus" could yield an output of 15 liters of blood per minute. Figures 3; references 4: 1 Russian, 3 Western.
[126-7813]

UDC: 616.22-008.57-07:681.31

QUANTITATIVE ESTIMATE OF HOARSENESS BY COMPUTER

Moscow VESTNIK OTORINOLARINGOLOGII in Russian No 2, Mar-Apr 82
(manuscript received 22 Oct 81) pp 41-44

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[Abstract] The authors have developed an automatic method for quantitative evaluation of hoarseness and for its application in clinical practice. The acoustical characteristic of hoarseness, manifested as instability (aperiodicity) of the base tone in terms of frequency was used as the parameter in the estimation. The method of investigation is that the test subject pronounces a vowel at a convenient tone and loudness, and the glottograms produced are used to analyze the "hoarseness coefficient," mathematically representing the dispersion of periods of the signal on the glottogram. This method of quantitative evaluation of hoarseness has been particularly useful in individual cases of examination of patients during the preoperative period and the postoperative period after endolaryngal microsurgical operations. Figures 1; references 19: 3 Russian, 16 Western.
[131-6508]

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SIGNIFICANCE OF MINUTE VOLUME PARAMETERS TO EVALUATION OF VESTIBULAR STABILITY

Moscow VOYENNO-MEDITSINSKIY ZHURNAL in Russian No 8, Aug 81 pp 48-50

[Article by A. S. Gusarov, Lt Col Med Serv]

[Text] The importance of studying vestibular stability is obvious. Thus, according to the data of A. I. Vozhzhova and R. A. Okunev (1964), the number of individuals subject to motion sickness not infrequently constitutes 90%. We know from works dealing with external respiration in the presence of vestibular instability that there is impairment of rhythm and nature of respiration in such a state (M. P. Chusov, 1940; L. A. Radkevich, 1969; V. S. Fomin, M. N. Migulina, 1970, and others); there are changes in pulmonary ventilation and gas composition of alveolar air (V. K. Stepanov et al., 1973). However, respiratory function has not been sufficiently studied.

Our objective here was to study the dynamics of external respiration in subjects differing in vestibular stability when exposed to Coriolis accelerations. For this purpose, we recorded on a vestibular unit the respiration rate (RR), minute volume (MV), heart rate (HR) and EKG at all stages of the studies. The unit consists of an automatic electric vestibular chair, to which is connected a Fiziolog-3 instrument with Reservy unit, an MT 1016 digital printer and 12-channel magnetoelectric K 12-22 oscillograph. In the study of vestibulovegetative reflexes, we used a 5-min test for endurance of cumulative Coriolis accelerations (ECCA) with intermittent exposure (according to I. I.Bryanov, 1963).

We tested 100 men 22 to 35 years of age with healthy vestibular analyzer. The subjects were divided into three groups: there were 80 men in the group with high vestibular stability (0 grade vestibulovegetative reactions--VVR), 10 with moderate stability (VVR grade I-II) and 10 with poor tolerance of motion (VVR grade III). In all, we recorded more than 3000 vestibulovegetative reactions.

The results of the findings obtained with the ECCA test enabled us to determine that the first group of subjects endured the 5-min test without complaints or external vestibulovegetative manifestations. The second group presented moderate pallor, perspiration and nausea, mainly in the 3d-5th min before stopping and after stops. We observed marked autonomic reactions in the third group (pallor, cold sweat, nausea, vomiting) either immediately after stopping the first time, or after the second-third time. One subject

Dynamics of minute volume, respiratory and heart rate during test for endurance of intermittent accumulation of Coriolis accelerations (ECCA) in subjects differing in vestibular stability $\bar{x} \pm m$

Groups of subjects	Physiological parameters	Background	ECCA test									
			before stopping					after stopping				
			1 min	2 min	3 min	4 min	5 min	1 min	2 min	3 min	4 min	5 min	10 min
High vestibular stability (VS)	MV, ℓ/min	11.2±0.5	11.5±0.8	12.1±0.5	12±0.5	12.7±0.8	12.3±0.8	11.7±0.4	12.3±0.6	11.8±0.4	11.9±0.6	11.7±0.8	10.7±0.5
	RR/min	16±1.1	16.3±0.7	16±0.7	15.3±0.6	15.6±0.7	15.5±0.9	16.7±0.6	15.5±0.7	15.1±0.6	15.2±0.6	16.1±0.7	16.3±1.6
	HR/min	68±1.5	45.4±2.2	47±1.9	45±1.8	47±1.8	45.6±1.7	66.5±1.9	68.1±2.2	68±1.5	68±1.8	63.9±1.9	67.9±1.7
Average VS	MV, ℓ/min	9.7±0.9	16.8±1.4	16.6±1.6	21.5±2.4	20.3±1.7	18.8±2.5	8.5±1	8.4±0.8	7.6±0.9	7.3±1.2	6.3±2.1	9.8±0.8
	RR/min	15.4±1	17.4±1.2	16.4±1.3	19.4±2.5	18.9±2	16.3±1.4	16.8±1.4	14.9±1.8	12.3±2.1	13.7±1.2	11.3±2.1	15.8±1.4
	HR/min	80±2.7	50.7±4.3	47.8±3.8	55.6±4.9	54.5±7.9	46±8.5	83.1±4.7	81.6±5.4	82.4±6.9	77.8±5.7	69.7±5.2	74.4±2.8
Low VS	MV, ℓ/min	11.2±1.3	19.5±2.2	19±1.3	14.1±1.2			8.3±1.7	6±0.8	4.3±0.6	5.2±0.7	6.1±1.4	8.9±0.8
	RR/min	15.2±0.9	17.8±1.1	18.2±0.6	17.3±1.3			13.2±0.7	12.9±1.3	12.2±1.7	12.8±0.8	12.4±1.3	14.2±1.8
	HR/min	76.9±1.6	66.6±6.9	55.8±6.1	68±6.9			81.7±3.4	77.3±4.7	85.8±6.7	81.4±5.8	78.1±4.6	76.4±3.7

developed vomiting after two stops without external vestibulovegetative manifestations. He was troubled by headache, vertigo and weakness. There was no change in the color of his face. The recovery period lasted more than 30 min.

In addition, there were rather distinct differences in respiratory system reactions of subjects differing in resistance to vestibular stimuli (see Table).

As can be seen from this table, RR showed virtually no change from the base level at all periods of rotation in the first group of subjects. Minute volume exceeded the base level insignificantly, by a mean of 1-2 ℓ/min. The heart rate decreased by an average of 22 beats/min.

After the first stop, there was some increase in RR (by 1-2/min), it slowed down after subsequent stops, also by a mean of 1-2/min. At all postrotation periods, MV exceeded the base level by a mean of 1-1.5 ℓ/min. Recovery of respiration and MV occurred 3-5 min after stopping. The heart rate was virtually at the base level at all postrotation periods.

In subjects with moderate vestibular stability (second group), the respiratory system reacted differently to the ECCA test. As can be seen in the table, pulmonary ventilation increased in the 3d, 4th and 5th min of rotation. MV constituted 18.8-21.5 ℓ/min (versus the base level of 9.7 ℓ/min) at the time of manifestation of grade I-II VVR. The heart rate had increased in the subjects prior to the test due to excitement, then decreased by a mean of 25-30 beats/min during rotation. After each stop there was gradual decline of minute volume from 8.5±1 to 6.3±2.1 ℓ/min ($P<0.001$). Respiration rate increased by 1-2/min in the first minute of rest, then slowed down by an average of 2-3/min in each period following rotation.

In the rest periods before the third stop, the heart rate exceeded somewhat the base level, then decreased by 2-10 beats/min. The period of recovery of MV, respiratory and heart rate lasted up to 5 min.

In the third group of subjects, with manifestation of motion sickness, minute volume increased significantly at all stages of rotation, against a background of slightly higher respiration rate, but with onset of nausea and just prior to vomiting, subjects who usually tried to suppress this sensation by holding their breath presented a decrease in MV. The mean data on heart rate are not always demonstrative. At the time of development of nausea, the rate of some subjects went up to 90-120/min.

In each rest period after stopping, minute volume decreased: to 8.3 ℓ/min after the first stop, 6 ℓ/min after the second and 4 ℓ/min after the third. This was associated with some decrease in respiratory rate, whereas heart rate increased. Further testing was usually discontinued due to marked vestibulovegetative manifestations. The period of recovery of MV, respiratory and heart rates lasted over 5 min.

Thus, these studies show that with development of vestibular instability some changes occur in external respiratory function, which is virtually not observed in healthy subjects. In individuals with heightened sensitivity to motion, hyperventilation appeared under the influence of Coriolis accelerations prior

to stopping, which was manifested by an increase in MV (to an average of 19 ℓ/min); after stopping we observed gradual decline by an average of 3-7 ℓ/min in pulmonary ventilation at all rest periods. There was slower recovery of MV, respiratory and heart rate in subjects with low vestibular stability than those with high vestibular stability.

Conclusions

1. In the presence of vestibular instability there is a change in reaction of the respiratory system to vestibular stimulation. In all periods prior to stopping, MV increases under the influence of Coriolis accelerations, and after stopping it presents a tendency toward decreasing.

2. Vestibulospirometry and vestibulospirography during and particularly after rotation are objective methods of recording vestibular stability of subjects.

3. Determination of dynamics of minute volume, with consideration of other autonomic reactions, could be used in medical certification of pilots for objective evaluation of vestibular stability of subjects.

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PHASE ANALYSIS OF DYNAMICS OF GALVANIC SKIN RESPONSES IN MAN

Riga IZVESTIYA AKADEMII NAUK LATVIYSKOY SSR in Russian No 2, Feb 81
(manuscript received 27 Jul 80) pp 123-125

[Article by A. A. Krauklis, A. A. Aldersons and I. A. Spandega, Latvian Scientific Research Institute of Experimental and Clinical Medicine, Latvian Ministry of Health]

[Text] One of the most frequently used parameters of dynamics of neuropsychological tension in man is the galvanic skin response (GSR) in the area of psychophysiological research [1-3]. Changes in activity of sweat glands, which cause a change in difference in electric resistance and bioelectric potential between skin regions under the measuring electrodes, are the main condition under which GSR occurs and presents dynamics [4, 5]. GSR appears in response to novelty or a stress stimulus due to intensification of psychogenic or emotional perspiration (with latency period of 1-3 s) [2, 4, 5]. As a rule, the dynamics of the GSR are studied by means of electrodes that are placed on the palms or, much less often, soles. It is generally believed that it is difficult or impossible to demonstrate a GSR in other parts of the body surface.

At the same time, it should be noted that there have been virtually no studies of the conditions and patterns determining the intensity and localization of GSR on the body surface and, in particular, selective localization of marked GSR on the palms and soles. Also unstudied is such an extremely important question for interpretation of GSR dynamics of factors that determine the paradoxical behavior of GSR in the presence of different intensities of neuropsychological tension: with moderate intensities of such tension the GSR is manifested in a marked form, whereas with high and low intensities there is minimal or no manifestation.

In this regard, we must stress that, in spite of the direct link between GSR and perspiration, the effect of temperature on formation of GSR has been studied very little.

Our objective was to determine the influence of the heat factor and, in particular, ambient temperature on appearance and dynamics of GSR in different parts of the human body both in a state of relative rest and with graded mental-emotional and physical loads.

Conditions of appearance and characteristics of phases of GSR in man

Factors	Dynamic GSR phases	Quantitative and qualitative characteristics of GSR
Laboratory situation determining initial (background) state of the subject	1	Observed exclusively on palms and soles
Start of exposure to graded stressor (temperature, physical factor, mental-emotional load)	2	Observed exclusively on palms and soles, showing gradual accentuation
Continued exposure to stressor	3	Starts to appear over entire surface of body
	4	Becomes synchronous and gradually increases over entire body; at the same time it diminishes significantly (reciprocal inhibition) on palms and soles
	5	Acquires a marked rhythm (brain tension rhythm of 0.1-0.3 Hz) and continues to increase synchronously reaching a maximum; as before, GSR on palms and soles is significantly diminished, there is drastic decline of GSR to exogenous stimuli
Termination of exposure to stressor	6	GSR gradually reverts to the first phase, undergoing phases 5, 4, 3 and 2 in reverse order

The studies were pursued on 1140 essentially healthy subjects ranging in age from 18 to 86 years, with 642 women and 498 men. We used the 13-channel Nihon Kohden encephalograph with a time constant of 1.5 s to record the physiological parameters. We made polygraphic simultaneous recordings of GSR from eight points of the integument following a specific protocol in each session. In all, we tested 86 points, 44 of which were on the limbs, 28 on the trunk and 14 on the head.

As a rule, we recorded the palmar GSR in each test. The GSR was recorded by the method of determining potential, as well as resistance of skin. The subject was in a shielded chamber in supine position. In the background period ambient temperature was 18-19°. We conducted tests with rapid elevation of temperature to 28-29° and with the use of loads: mental-emotional (solving arithmetic problem), emotional (subjectively unpleasant electrodermal

stimulation), static physical load (squeezing rubber bulbs applying 60% of maximum force for 50 s) and dynamic physical exercise (20 squats). Concurrently with polygraphic recording of the GSR, we recorded the electrocardiogram, electromyogram, pneumogram and arterial pressure in order to assess the dynamics of intensity of psychoemotional stress.

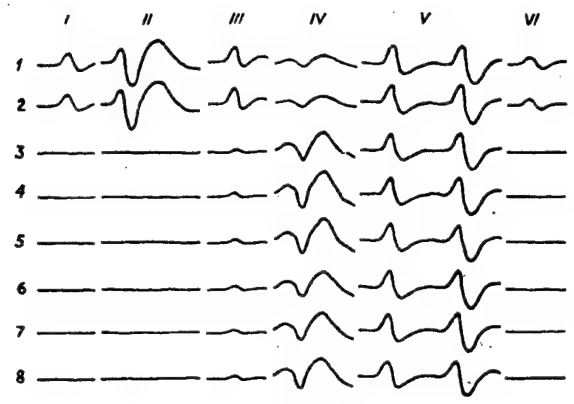

Figure 1.
Tracings of phases of human GSR on polygraphic recording:

I, II, III, IV, V, VI) phases of GSR dynamics
1) GSR from left palm
2) from right palm
3) from left forearm
4) from right forearm
5, 6) from trunk
7) from head

It was established that GSR dynamics undergo five phases with rapid elevation of temperature (see Table). In the base state, a GSR is observed only on the palms, and it begins to increase under the influence of elevation of ambient temperature. With continuation of exposure to high temperature, the GSR starts to appear over the entire surface of the body, becomes synchronous and the process of reduction of GSR on the palmar surfaces begins (reciprocal inhibition). At the next stage of development, GSR acquires a distinct rhythm of 0.1-0.3 Hz; at the same time there is drastic reduction or complete disappearance of GSR to exogenous stimuli. When ambient temperature reverts to the base level (18-19°), the GSR dynamics undergo the above-mentioned phases in reverse order back to the first phase (Figure 1). At a temperature of 18-19°, rather intensive physical or emotional loads could cause appearance of the second and third stages, less often the fourth and fifth as well, without raising temperature in the room. The same loads could also cause rapid change to any of the next stages with exposure to elevated temperature. It should be noted that our special studies of patients with visceral pathology revealed that the main phases of the GSR undergo the same stages as healthy subjects, the only exception being segments corresponding to the involved organ, where the dynamics are drastically altered.

Thus, our findings enable us to conclude that the dynamics of development of GSR present distinct phases, which depend on ambient temperature, emotional and physical tension.

The decrease in intensity of GSR of the palms and soles observed in the fourth phase is usually associated with increased intensity of GSR in all other parts of the skin, which warrants the assumption that reciprocal relations appear between nerve centers regulating perspiration of the palms and soles, on the one hand, and centers regulating perspiration of the rest of the skin surface, on the other.

The results of this study indicate that phase analysis of GSR dynamics increases the informativeness of this parameter substantially, and that it is only with consideration of the GSR phases and concrete conditions of appearance of the distinguished phases that it is possible to adequately interpret GSR dynamics.

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USE OF GSR PHASE ANALYSIS METHOD FOR HIGH-SPEED DIAGNOSIS OF VISCERAL PATHOLOGY

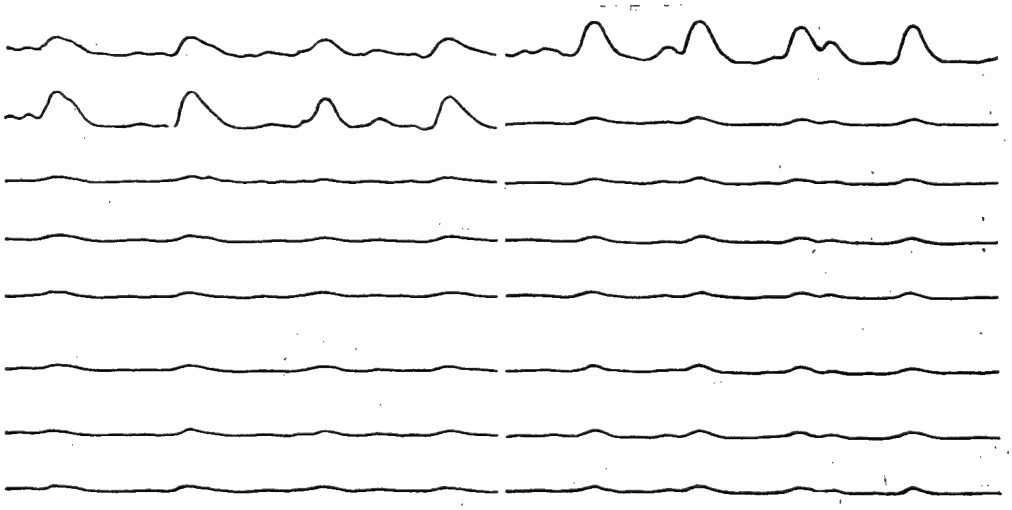
Riga IZVESTIYA AKADEMII NAUK LATVIYSKOY SSR in Russian No 2, Feb 81 (manuscript received 4 Dec 80) pp 126-129

[Article by A. A. Aldersons and A. A. Krauklis, Latvian Scientific Research Institute of Experimental and Clinical Medicine, Latvian Ministry of Health]

[Text] Physicians have long since established that heightened sensibility and tenderness of specific parts of the body surface are often observed in the presence of visceral pathology. The studies of G. A. Zakhar'in [3], Head [4] and B. I. Vil'yamovskiy [1] have demonstrated the existence of a reflex connection between internal organs and specific parts of the skin, so-called reflexogenic zones which are notable for heightened sensibility and reactivity in the presence of diseases of the corresponding internal organs. It was learned that drastic changes in electric potential and resistance of the skin appear in the above zones via the reflex pathway in the presence of visceral pathology. Numerous authors have tried to make use of this pattern for the purpose of electrodermal high-speed diagnosis of visceral pathology. However, these attempts failed, since the methods used were generally based on recording and evaluating the dynamics of only slow potentials or only slow oscillations of electric resistance of skin, which change continuously and markedly under the influence of exogenous factors and background fluctuations in functional state of the nervous system and entire body, so that they cannot assure stable and comparable data, let alone reliable criteria of "normal" and "pathology." Moreover, judging by the methods described in the literature, studies of electrodermal effects were usually pursued without using adequate functional loads on the subjects--physical, mental and emotional, thermal--which reduces appreciably the possibility of detecting pathological changes in electrodermal potentials in the Zakhar'in-Head zones.

In order to overcome the above-mentioned methodological flaws, we have developed a method of phase analysis of electrodermal, or galvanic skin response (GSR), based on detection of the rapid component of electric potentials, or electrodermal resistance of the tested skin zones, as well as quantitative determination of changes in this component under the influence of standard graded loads [2]. Our objective here was to determine whether this method can be used as a reliable diagnostic test on patients for high-speed detection of visceral pathology.

This study was conducted on 370 patients (200 women and 170 men), 122 of whom had gastrointestinal pathology (peptic ulcer, exacerbation of chronic cholecystitis, gastritis, acute appendicitis, etc.), 86 presented cardiovascular pathology (angina pectoris, myocardial infarction), 90 had genitourinary pathology (inflammation of the bladder and ovaries, bladder calculi) and 72 had lung pathology (pneumonia and others). We made a polygraphic recording of GSR from eight zones of the skin simultaneously: six zones corresponded to the Zakhar'in-Head zones of suspected pathology, one zone on the palms and distal part of the forearm served as a control; we also recorded the EKG, pneumogram, arterial pressure and EMG (extensors of the fingers). The study was conducted with rapid graded elevation of temperature in the laboratory from 18 to 29°. In addition to the heat load, we also used standard graded mental-emotional and physical ones (visual retrieval with limited time allowance, anticipation of probable electrodermal stimulation, manual dynamometry (squeezing rubber bulbs for 30 s at 60% of maximum force, squats and step test in a specified rhythm). The physiological parameters were recorded on a 13-channel recording instrument of the Nihon Kohden firm.



Polygraphic tracing of GSR of patient with exacerbation of chronic peptic ulcer. Left--before course of therapy; right--after completing therapy course. Location of measuring electrodes (top to bottom): left palm; reflexogenic zone of stomach; duodenum; gallbladder; lungs; heart; urinary bladder and control zone

It was established that with the use of the above loads the GSR underwent the same five phases that we had demonstrated in healthy subjects and described in our previous work [2]. However, unlike the healthy subjects, the third phase of GSR dynamics was manifested in patients first and considerably more in the Zakhar'in-Head zone, which corresponds to localization of visceral pathology. As the GSR progressed to the next, fourth phase, the exceptional intensity of GSR in the "pathological" reflexogenic zone gradually decreased and became the same as the intensity of GSR in other tested skin areas (Figure). Thus, the diagnostic value of GSR is the most marked at the third phase, when one can reliably and very accurately detect and localize the

pathological site in some visceral system on the basis of time and quantitative features of GSR dynamics (see Table).

Characteristics of GSR phases in patients with visceral pathology

Factors to which the patient is exposed	GSR phases	Quantitative and qualitative GSR characteristics
Laboratory situation determining the patient's initial (background) state	1	GSR observed exclusively on palms and soles
Start of graded stressor (temperature, physical, mental-emotional) load	2	GSR observed exclusively on palms and soles, and it gradually increases
Continued exposure to stressor	3	a) GSR starts to appear in reflexogenic zones corresponding to involved organ b) GSR increases in zones corresponding to involved organ and starts to appear in reflexogenic zones and biologically active points corresponding to system of organs c) GSR increases in reflexogenic zones and biologically active points corresponding to given system of organs, and it is diminished on palms and soles d) GSR starts to appear over entire body surface
	4	GSR increases over entire body surface and is inhibited on palms and soles
	5	GSR acquires a pronounced rhythm, increasing synchronous to a maximum. Drastic decline of GSR to exogenous stimuli
Termination of stressor load	6	GSR gradually reverts to phase 1, undergoing phases 5, 4, 3 and 2 in reverse order

In order to make an accurate quantitative evaluation of intensity of reflex involvement of reflexogenic zones in the pathological process in internal organs, the dynamics of GSR in control zones are very important. According to findings made on 1140 healthy subjects [2], symmetrical distal parts of the forearms are the most suitable control zones, since GSR changes when passing from one phase to the next one in these zones usually occur later than in other parts of the skin. For this reason, it is convenient to coordinate determination of GSR in the tested skin zones with the time that the GSR of the control zone reaches a very definite value. In our

study, we used the time when GSR, expressed in units of electric resistance, reached 1 kΩ in the control zone. We also determined the ratio of GSR in the tested Zakhar'in-Head zones to GSR in the control zone. Normally, this relationship equals 1 at all phases of GSR, including the third one. Thus, in healthy man, the correlation between GSR of different skin zones at the third phase is expressed by the formula, 1:1:1:1, etc.:(1), where (1) is the unit of GSR in the control zone. In patients, this correlation is drastically altered due to intensification of GSR in the zones corresponding to the involved organ. For example, in patients with peptic ulcer, the correlation between GSR in reflexogenic zones of the stomach, kidneys, bladder, heart, lungs and control zone in the third phase is often expressed by the formula, 3.4 or 5:1:1:1:1:(1). As a result, the method we developed enables us to compare and evaluate quantitatively the data on GSR dynamics in different zones of the body surface of the same patient, as well as to compare and evaluate GSR data of different patients.

Example: Patient A. V., 56 years old, a teacher. Clinical diagnosis: ischemic heart disease. Atherosclerotic coronary cardiosclerosis. Tension angina pectoris. Cardiovascular insufficiency, grade 2b. Chief complaints: pain in the cardiac region when tense, excited and fatigued. The ratio of GSR in the tested skin zones--reflexogenic zones of the heart, stomach, duodenum, gallbladder, lungs, bladder and two control zones in distal parts of the forearms--was 2:1:5:1:1:1:1:1. Additional examinations confirmed the objectivity of the suspected duodenal pathology. Conclusion from pH measurement of stomach: partially compensated atropine-areactive, markedly acid stomach. Conclusion of roentgenoscopy of the stomach and duodenum: duodenal ulcer.

Clinical and physiological observations have shown that the results of polygraphic and polymetric phase analysis of GSR dynamics with the above-mentioned loads can serve as a highly sensitive and adequate indicator of functional changes in involved organs under the influence of a course of treatment, which permits reliable evaluation of efficacy of therapeutic measures and forecasting the dynamics of the disease during the period following a course of treatment of the patients at a clinic, sanatorium or polyclinic. In view of the foregoing, it can be concluded that phase analysis of GSR dynamics is a promising ancillary method for high-speed detection of visceral pathology in medical practice, which can facilitate appreciably the detection of latent pathology of internal organs, as well as permit evaluation and forecasting of the dynamics of pathology before, during and after a course of therapy.

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BLOCKING ACTION OF ETHIMIZOL, CADMIUM IONS AND TEA ON IONIC CURRENTS IN ISOLATED NEURONS OF LIMNAEIDAE

Moscow DOKLADY AKADEMII NAUK SSSR in Russian Vol 262, No 4, Feb 82
(manuscript received 1 Sep 81) pp 1017-1021

ZAYTSEV, Yu. V. and VISLOBOKOV, A. I., Scientific Research Institute of Experimental Medicine, USSR Academy of Medical Sciences, Leningrad; Leningrad State University imeni A. A. Zhdanov

[Abstract] In the present study comparative data on the effect of ethimizol, Cd^{2+}, and TEA on the ionic currents of limnaeidae neurons were obtained using the method of intracellular dialysis and fixation of the membrane potential of isolated neurons. This method made it possible to register individual ionic currents under conditions where various substances could exert their effect from the internal or external side of the membrane. Since ethimizol showed no effect on the ionic currents in dialyzing solution, only perfusing solutions were used in this study. The primary effect of ethimizol was due to its interaction with external components of the plasma membrane. These components could be identical with those observed with Cd^{2+}. They don't have to be specific in the sense assumed for chemoreceptors reacting with mediators. The effect of ethimizol on the ionic currents of the electroexcitable membrane of the neurons is characterized by polymodality with predominant depression of rapid potassium currents and considerable lowering of the conductivity of other ionic channels.
Figures 2; references 15: 12 Russian, 3 Western.
[124-7813]

UDC: 577.391:621.039.58

INFORMATION ABOUT ALL-UNION WORKING CONFERENCE ON 'THEORETICAL BASES OF PROTECTION AGAINST RADIATION AND PRINCIPLES INVOLVED IN SEARCHING FOR NEW RADIOPROTECTIVE AGENTS'

Moscow RADIOBIOLOGIYA in Russian Vol 21, No 5, Sep-Oct 81 pp 796-797

[Article by M. M. Konstantinova and E. A. Tarakhtiy]

[Text] The All-Union working conference on "Theoretical Bases of Protection Against Radiation and Principles Involved in Searching for New Radioprotective Agents," convened in Sverdlovsk on 25-26 November 1980; it was organized by the Section "Protection Against and Recovery From Radiation Damage" of the "Radiobiology" Scientific Council of the USSR Academy of Sciences, Institute of Developmental Biology imeni N. K. Kol'tsov, USSR Academy of Sciences, and Institute of Plant and Animal Ecology, USSR UNTs [Ural Scientific Center]. The conference was dedicated to the memory of the outstanding Soviet radiobiologist, Prof Emanuil Yakovlevich Grayevskiy, doctor of biological sciences. More than 60 researchers participated in this conference, from various cities of our country, who work in the field of synthesis and selection of new radioprotective agents, and investigation of the mechanism of their action.

In his opening remarks, A. G. Sverdlov (Leningrad), chairman of the Scientific Council section, mentioned the importance of the topic of the conference and major contribution made to the development of radioprotection theory by E. Ya. Grayevskiy.

M. M. Konstantinova (Moscow) dealt with the life and creative achievements of E. Ya. Grayevskiy, a remarkable Soviet scientist, pedagogue and human being. A. G. Sverdlov analyzed the inception and current status of the "sulfhydryl hypothesis" expounded by E. Ya. Grayevskiy, demonstrating its significance to the formation and development of conceptions concerning factors that determine systemic and cellular radioresistance.

Much attention was devoted at this conference to reports on the results of the search for new compounds with radioprotective action among various classes of chemicals.

In recent years, there were intensive studies of various isothiuronium derivatives, among which the most promising are salts of ethyl and isopropyl isothiuronium, phosphoric and metaphosphoric acids (Zh. A. Goloshchapova, Chelyabinsk). Substances similar in activity to AET were found among quinazoline

and pyrimidine derivatives of isothiuronium; for this category of compounds, a link was demonstrated between activity and their structure (B. V. Golomolzin, Sverdlovsk). Some interesting data were obtained on radioprotective properties of a less-studied class of compounds--thiazole and benzothiazole derivatives (A. P. Novikova, Sverdlovsk). It was demonstrated that unadulterated and synthetic porphyrins are capable of altering radiosensitivity of normal and tumor tissues (Ye. I. Yartsev, Moscow), as well as laboratory animals (S. M. Puchkova and T. N. Tuzhilkova, Chelyabinsk). Some highly active compounds were found among derivatives of tetrazole (V. G. Kitayeva, Sverdlovsk) esters of dimethyl thiazolidine of carboxylic and mercaptopropynoic acids (S. M. Puchkova, Chelyabinsk), which are effective when administered by different routes. It was reported that cations affect toxicity and radioprotective efficacy of salts of S-(2-aminoethyl) thiophosphoric acid (V. Yu. Kovtun, Moscow). Condensation of radioprotective agents with colchicine leads to appearance in this agent of condensation of radiosensitizing properties (A. I. Begisheva, Tashkent).

The proceedings of the conference contained data on the effects of radiation and radioprotectors on renal function (Ye. I. Sukhacheva, Sverdlovsk), reticuloendothelial system of the spleen, hemopoietic tissue, intestinal and spermatogenic epithelium (Sverdlovsk), activity of K^+, Na^+-ATPases of organs differing in radiosensitivity and on metabolism of acetylcholine in nerve tissue (Dnepropetrovsk).

The delivered papers dealt with a number of basic questions of radioprotection. It was noted that, in assessing the efficacy of protective agents, one must take into consideration, first of all, their concentration in the cell, which is determined by the physicochemical properties of the protective agent and pH gradient between the cell and medium (A. M. Veksler, Pushchino). A comparison was drawn of the informativeness of various models proposed for evaluating the efficacy of radioprotective agents and investigating the mechanism of their action: cellularity of bone marrow, yield of chromosomal aberrations in animal cells and bone marrow chimera (rat--mouse), cloning of bone marrow stem cells in vivo and in monolayer culture, intensity of assimilation of citric acid, etc. (V. V. Kozhevnikov, Perm'). It was noted that, in assessing the efficacy of protective agents on the cellular level, consideration must be given to the stage of the cell cycle at which the study is made (N. A. Poryadkova, Obninsk), whereas in assessing the radioprotective effect on the systemic level one should not limit oneself solely to determining its influence on number of surviving CFU [colony-forming units], as indicated by comparing survival and CFU (exotest) of animals irradiated after being given a protector (serotonin) (G. V. Dontsova, Moscow). The paper of L. M. Rozhdestvenskiy (Moscow) stressed the importance of determining the main protective factor, identify of experimental conditions and organic localization of radioprotective agents when assessing their pharmacological and radioprotective effects. This author believes that changes in cellularity of bone marrow, which reflect the degree of protection and, consequently, survival, are among the most informative indicators of the radioprotective effect. A correlation between hypoxic and protective effects of mexamine and catecholamines was demonstrated on this model. Chemical and, perhaps, enzymatic repair are the factors that effect protection.

Several objections were raised to the mathematical model of radiation damage and radioprotection submitted by A. S. Pogorelov (Khar'kov).

The conference devoted attention to the mechanism of action of protective agents. There was discussion of the mechanism of radioprotection action of aminoalkyl isothiourea, the efficacy of which is related by authors to their change into heterocyclic compounds in the body (A. A. Mandrugin, Moscow). Apparently, indene compounds and pigments (melanin) that lower the incidence of spot mutations act as "energy traps" (I. B. Mosse, Minsk). Studies of the link between thiol content and level of antioxidant activity of lipids in the same organ of the same animal failed to demonstrate the expected correlation, whereas there is one between mean group values of these parameters (G. F. Ivanenko, Moscow).

One of the most important tasks put to researchers by clinicians is to increase the efficacy of protectors and the range of protection. O. G. Zherebchenko (Moscow) reported on advances in this direction. Use of a 7-component combination of substances increases the DRF [dose reduction factor] to 2.8, whereas the most effective of the components used in the mixture yields a DRF of 2. The author assumes that the limited number of "receptors," interaction with which is needed for expression of activity, is the reason for a limit to the protective effect. It is theorized that even minor changes in molecular structure of a protective agent enables it to interact with other "receptors."

It was demonstrated that known (cystaphos, gammaphos, AET, cystamine), as well as new (pentaphos, thiogammaphos, thiocystaphos) sulfur-containing radioprotectors can be used in principle for effective protection against neutron radiation. Indolylalkylamines did not attenuate the neutron-induced damage; however, a combination of agents referable to the above-mentioned groups diminished the radiation damage to the critical system--the intestine. This opens the way for enhancing protection against neutrons by using combinations of protective agents (A. G. Sverdlov, Leningrad). It was noted that it is expedient to combine radioprotective agents with leukopoietins, trefons [?], antikeylons [?], which regulate the number of proliferative pools of bone marrow and intestinal epithelial stem cells determining systemic radioresistance (V. S. Korytnyy, Chelyabinsk).

In summing up the achievements of the conference, A. G. Sverdlov, P. G. Zherebchenko, M. M. Konstantinova and D. I. Semenov (chairman of the organizing committee, Sverdlovsk) noted that it was fruitful and that such conferences are necessary, since they make it possible to directly exchange new information and stimulate research on the problem under discussion.

The conference participants expressed their satisfaction with the good organization of the conference, hospitality and concern displayed by the residents of Sverdlovsk.

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EFFECTS OF LASER EMISSIONS ON HUMANS

Kiev VRACHEBNOYE DELO in Russian No 9, Sep 81
(manuscript received 9 Jan 81) pp 10-15

SUVOROV, I. M., DOBRYNINA, V. V., USHKOVA, I. N., LYCHAKOVA, L. N. and SUSHENTSOVA, T. I., Department of Occupational Pathology, Leningrad Scientific Research Institute of Labor Hygiene and Occupational Diseases

[Abstract] Soviet literature was surveyed for clinical observations on persons engaged in working with various types of lasers ranging in power from milliwatts to kilojoules. Combined with experimental findings, the results pointed to visual, neurological, hematological and biochemical sequelae, and emphasized the feeling expressed by many Soviet specialists of the need for national safety standards to protect individuals under occupational risk. References 43 (Russian).
[146-12172]

UDC 615.851:616.8-009.836.14:615.214.26:616.26:616.8-009.836.14-06

SLEEP DEPRIVATION IN PSYCHOTHERAPY OF INSOMNIA DUE TO PSYCHIC DEPENDENCE ON SOPORIFICS

Kiev VRACHEBNOYE DELO in Russian No 3, Mar 82
(manuscript received 2 Jun 81) pp 104-106

LITVINENKO, V. I. and KRYLOVSKIY, A. P., Geyko Oblast Psychiatric Hospital of Dnepropetrovsk Oblast

[Abstract] Sleep deprivation has been tested as a therapeutic modality in restoring natural sleep in soporific-dependent mentally-disturbed patients. Studies with 29 female schizophrenics deprived of sleep for 24 h at two-day intervals for two to six courses of therapy demonstrated the efficacy of this approach in that 19 subjects did not resort to drug-induced sleep for a year after treatment. The control group was composed of 31 women with clinical schizophrenia; of these, 27 continued to require soporifics to induce sleep. The combination of sleep deprivation and subsequent compensatory sleep was felt to be the key mechanism in breaking the conditioned reliance on sleep-inducing drugs. References 3 (Russian). [236-12172]

SYSTEMIC ORGANIZATION OF EMOTIONAL BEHAVIOR

Moscow USPEKHI FIZIOLOGICHESKIKH NAUK in Russian Vol 13, No 2, Apr-Jun 82 pp 123-126

BELYY, V. P.

[Review of "Sistemnaya organizatsiya emotsional'nogo povedeniya" [Systemic Organization of Emotional Behavior] by Yu. A. Makarenko, Moscow, Izdatel'stvo "Meditsina", 1980, 207 pp]

[Abstract] The book here reviewed is dedicated to the experimental study of various physiological indices of the animal body when emotional states of directly opposite nature are created in them by electric stimulation of

various hypothalamic nuclei: the state of positive reinforcement for satisfaction and the state of negative reinforcement for dissatisfaction. The monograph includes an introduction and 8 chapters. The first two chapters present reviews of existing conceptions concerning the mechanisms of motivation and reinforcement, plus information on the topography and neurochemistry of the satisfaction centers and punishment centers in the brain. Subsequent chapters present results of the author's experimental studies. Chapter 8 presents general conclusions concerning the characteristics of various positive reinforcement effects. The book is criticized by the reviewer for shooting at too broad a target, so that some aspects are not thoroughly, experimentally developed. However, it is considered a valuable text for specialists in the area of the neurophysiology of behavior, motivation and emotion, problems of cybernetic modeling of complex adaptive systems and pathological psychology.
[223-6508]

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